THE UNIVERSITY OF KANSAS
SCIENCE BULLETIN

VOL. XVI.] MARCH, 1926. [No. 1.

Antigenic and Metabolic Studies of *Bacillus typhosus*.*

CORNELIA M. DOWNS, Department of Bacteriology.

CONTENTS.

PAGE

INTRODUCTION 6

PART I. CULTURAL AND SEROLOGICAL REACTIONS 7

EXPERIMENTAL:

Experiment 1. Cultural Reaction 7

Experiment 2. Serological Reactions 11

Methods:

Agglutination Tests 11

Absorption Tests 11

Widals 12

RESULTS 9, 13

DISCUSSION 18

CONCLUSIONS 22

PART II. METABOLIC STUDIES 23

EXPERIMENTAL:

Purpose 23

Methods and Technique 23

QUALITATIVE TESTS. *Effect of Hydrogen-ion Concentration:*

Experiment (*a*). Range of hydrogen-ion concentration at which growth occurs 24

Experiment (*b*). Changes in hydrogen-ion concentration in plain broth 28

Experiment (*c*). Final hydrogen-ion concentration in broth containing various fermentable carbohydrates 29

Experiment (*d*). The viability of *B. typhosus* in broths containing a fermentable carbohydrate 36

Experiment (*e*). The influence of varying factors on the final hydrogen-ion concentration in dextrose broth 37

DISCUSSION 37

SUMMARY 48

QUANTITATIVE TESTS. *Dextrose Metabolism and Correlated Factors* 49

Review of literature 49

Methods and technique 52

Experiment (*a*). Utilization of dextrose and influence of buffers 53

Experiment (*b*). Rate of acidity formation 59

Experiment (*c*). Growth rates 67

Experiment (*d*). Rate and relation of the utilization of dextrose to final hydrogen-ion concentration and titratable acidity, inhibition and death 68

DISCUSSION 70

SUMMARY 74

DISCUSSION OF IRREGULAR STRAINS 75

GENERAL DISCUSSION 79

GENERAL SUMMARY AND CONCLUSIONS 84

BIBLIOGRAPHY 86

ACKNOWLEDGMENT 89

* Submitted to the Department of Bacteriology and the Faculty of the Graduate School of the University of Kansas in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

INTRODUCTION.

RELIABLE methods for the identification and classification of bacteria have been eagerly sought by many investigators. Kendall (1923) says, "We are not so much interested in what bacteria are as in what they do." Morphological classification has been largely superseded by physiological classification. It has been found that the cultural reactions of bacteria are fairly stable and that most organisms can be divided into groups by means of these reactions. As the science of immunology has developed it has been found that the members of these groups, although giving identical cultural reactions, can be separated into subgroups by means of serological tests. A case in point is that of the pneumococcus group. All pneumococci give certain definite cultural reactions, but serological tests separate these apparently identical organisms into four sharply defined groups. Of late years studies on streptococci, staphylococci, *B. dysenteriæ*, *B. diphtheriæ* and others have shown that organisms may be grouped by means of cultural reactions and these groups further subdivided by serological reactions.

The stability of these cultural and serological reactions has an important bearing on their use in the identification of organisms. Variations in physiological and antigenic properties of many organisms have been reported.

The typhoid group has usually been considered homogeneous in cultural and antigenic reactions, and if this were true it would seem to differ sharply from the majority of other organisms. Enough variation, however, has been reported by Hooker (1916), Weiss (1917), Teague and McWilliams (1919), Rotky (1921), and others, to give point to an intensive study of several strains of *B. typhosus*. This work was accordingly undertaken.

Part I deals with a study of the antigenic differences exhibited by strains of *B. typhosus* and the relation of such antigenic differences to any cultural peculiarities present.

Part II deals with the effect of hydrogen-ion concentration on the metabolism of these same strains; their dextrose metabolism and viability, and the correlation of these reactions with their antigenic characters.

PART I.

CULTURAL AND SEROLOGICAL REACTIONS.

EXPERIMENT 1.—CULTURAL TESTS.

METHODS: Thirty-two strains of *B. typhosus* were collected from as widely separated sources as possible. The source, place of isolation, name and date are shown in Table I. Preliminary cultural reactions were determined on each strain. The carbohydrate medium was Hiss' semisolid medium, to which was added 1 per cent of the carbohydrate desired and Andrade indicator to make a pale flesh color when cold. A duplicate set of determinations was made, using meat-infusion broth, adjusted to pH 7.0, to which 1 per cent of the carbohydrate was added, litmus being used as an indicator. For the lead-acetate agar 1 cc. of 1 per cent lead-acetate solution was added to the semisolid medium. To test for liquefaction and gas production on noncarbohydrate medium, a 2 per cent peptone gelatine, made according to Treece (1920), was used. The gram strains were made from 24-hour meat-extract broth cultures, and also from 24-hour agar slants.

TABLE I.—Strains of *B. typhosus* used.

1	Blood culture, Lawrence, Kan.	57	1913
21	Blood culture, Kansas City, Mo.	.....	1919
23	Blood culture, University of California.	.....	1914
25	Blood culture, Johns Hopkins Hospital.	.....	.....
27	Blood culture, Kansas City, Mo.	Nelson	1919
36	Blood culture, Lawrence, Kan.	Brownbeck	1920
40	Blood culture, Lawrence, Kan.	Clements	1921
4	Feces, Lawrence, Kan.	Smith	1919
5	Feces, Lawrence, Kan.	Hunter	1919
6	Feces, Lawrence, Kan.	.....	1918
8	Feces, Lawrence, Kan.	.....	1919
16	Feces, carrier, Beau Desert, France	.....	1918
20	Feces, Topeka, Kan.	.....	1919
24	Feces, fatal case, Johns Hopkins Hospital	.....	1919
30	Feces, carrier, Michigan	Dardrich	1920
31	Feces, carrier, Michigan	.....	1920
32	Feces, carrier, Michigan	Dowd	1920
33	Feces, carrier, Michigan	.....	1920
34	Feces, carrier, Michigan	.....	1920
39	Feces, case, Lawrence, Kan.	Hess	1921
44	Feces, case, Lawrence, Kan.	.....	1921
7	Spinal fluid, Halstead, Kan.	.....	1919
12	Spleen, autopsy	Rawlings	.....
15	Gall bladder, autopsy, France	Wahle	1918
2	.....	.....	.....
3	.....	Bender	.....
10	No history, New York Board of Health	Mt. Sinai	.....
11	.....	Pfeiffer	.....
13	American Museum	Hopkins	.....
14	American Museum	Miller	.....
17	Institute of Berlin	Ebert	1888
19	University of Chicago	Jordan	1889

RESULTS.

Table II shows that the majority of the cultural reactions were typical. Three strains—Nos. 21, 40, and 12 (the Rawlings strain)—fermented xylose slowly. Weiss (1917) has reported that the Rawling's strain does not ferment xylose. Teague (1920) considered this strain to be a slow fermenter. The litmus milk was kept six weeks before being discarded. The milk remained a permanent lilac color in all except six organisms. Five turned back to neutral in three weeks and one became a deep blue color after one week. Three strains gave slight acidity in salicin and two strains were negative in dextrin. Krumwiede (1918) reports fermentation in dextrin as varying with the sample used. The two cultures giving an alkaline reaction in dextrin might, therefore, have shown typical acid production in another sample. My strains were uniformly negative on dulcite and arabinose. Teague (1920) reported that eleven out of forty-one strains of *B. typhosus* fermented these sugars slowly. In addition to the above strains an organism isolated from the feces of a clinical case of mild typhoid was studied. This organism is designated as No. 5. The patient at no time gave a positive Widal. The organism differed culturally from *B. typhosus* in that it blackened lead acetate slowly, did not ferment xylose nor dextrin, fermented rhamnose, and gave a distinct alkaline reaction in litmus milk after seventy-two hours, but with no saponification.

The danger of confusing non-gas-producing paratyphoid strains with *B. typhosus* has been emphasized. Ten-Broeck (1916) reports a non-gas-producing hog-cholera bacillus which resembled in some respects *B. typhosus*. Krumwiede (1918) also reports a similarity in cultural and serological reactions between *B. pullorum* and *B. sanguinarium* and *B. typhosus*. Myers (1920) reports the isolation from a clinical case of typhoid of a rhamnose-positive *B. typhosus* which was also atypical in its serological reactions. It was difficult to decide, therefore, whether No. 5 was a true but irregular *B. typhosus* strain or a non-gas-producing paratyphosus. Krumwiede* kindly examined this strain, and using the fermentation of rhamnose as the deciding factor between the typhoid and paratyphoid groups, considered it a member of the latter group.

---

* Personal communication from Dr. Charles Krumwiede.

TABLE II.—Cultural characteristics.

NUMBER.	Litmus milk.	1 neutral after 1 week.		15, 17, 19 neutral after 3 weeks.		Acid after 10 days.	23, 24, 26 neutral after 3 weeks.		
1, 2, 3, 4, 6, 7, 8	Xylose	+	+	+	|	+	+	+	+
	Arabinose	|	|	|	|	|	|	|	|
	Dulcite	|	|	|	|	|	|	|	|
	Salacin	|	+	|	|	|	+	|	+
	Rhamnose	|	|	|	|	|	|	|	|
	Dextrin	+	+	+	+	+	+	+	|
	Saccharose	|	|	|	|	|	|	|	|
	Lactose	|	|	|	|	|	|	|	|
	Maltose	+	+	+	+	+	+	+	+
	Mannite	+	+	+	+	+	+	+	+
	Dextrose	+	+	+	+	+	+	+	+
	Lead acetate	+	+	+	+	+	+	+	+
	Indol	|	|	|	|	|	|	|	|
	Gelatine	|	|	|	|	|	|	|	|
	Gram	|	|	|	|	|	|	|	|
	Motility	|	|	|	|	|	|	|	|
9									
10, 11, 13, 14, 15, 16, 17, 20									
12									
21									
23–25									
24, 28, 29, 30, 31, 32, 33, 34, 35									
26, 27									

EXPERIMENT 2.—ANTIGENIC DIFFERENCES EXHIBITED BY DIFFERENT STRAINS OF B. TYPHOSUS.

Antigenic irregularities in *B. typhosus* have been noted by Stober (1904), Teague and Torrey (1907), Sheller (1909), Muller (1911), Raubitschek (1913), and Moon (1914). Recently Hooker (1916) and Weiss (1917) have offered a tentative grouping of typhoid strains based on antigenic differences. The occurrence of antigenic differences among several strains of *B. typhosus* used in diagnostic work in this laboratory had been noted from time to time and these differences served to indicate the advisability of an investigation of the serological characteristics of a number of strains of *B. typhosus*. The serological work herein reported consisted of a study of agglutination and absorption tests and Widals using the organisms given in Table I.

AGGLUTINATION TESTS.

METHODS. Eleven sera were prepared by the intravenous injection of killed cultures of *B. typhosus* into rabbits. This was supplemented by the injection of living cultures after several doses of killed cultures had been given. The titre of these sera ranged from 1-500 to 1-15,000. In addition, five sera were obtained from commercial laboratories: Parke-Davis serum, titre 1-10,000; Wichita laboratory, 1-400; University of Chicago, 1-10,000; Lederle (Rawlings, monovalent serum), 1-8,000; New York city board of health, 1-80,000 (bivalent serum prepared from the Mt. Sinai strain, and a freshly isolated strain). All agglutination tests were performed with living cultures washed from a 24-hour agar slant with sterile saline to make an even emulsion. Equal quantities of the suspension and the designated dilution of serum were added to clean test tubes. These tubes were incubated two hours in a water bath at 37° C., and readings were taken. Final readings were taken after twelve to fourteen hours in the ice box.

ABSORPTION TESTS.

The absorption tests were performed as follows: The serum to be absorbed was diluted to one-tenth its titre. This dilution was then saturated with the organisms washed from a 24-hour agar slant to make a heavy emulsion. This was incubated at 37° C. for four hours, and for four days at ice-box temperature, more organisms being added as the supernatant fluid became clear. This length of time was found to be necessary to give complete absorption. A control of diluted serum alone, without organisms, in every case gave a

good agglutination in spite of the prolonged incubation. A second control of the diluted serum, absorbed with the strain used for immunization, was used to determine if absorption were complete. We found the following technical points must be considered in any absorption test:

1. Weight of suspension.
2. Dilution of serum.
3. Time of absorption.
4. Repeated saturation.
5. Temperature.
6. Adequate controls.

Krumwiede (1918) recommends a proportion of one to four or three, or at most one to two, of packed cells to supernatant fluid. Our proportion after the final centrifuging was one to three. It was found that a dilution of one-tenth the titre of the serum was perfectly satisfactory. Although higher dilutions could be used, a lower dilution did not give complete absorption. Three or four hours was not long enough to give complete absorption and frequently absorption was not complete in twenty-four or forty-eight hours. After a standard of four days was chosen, no more trouble was experienced. It was always necessary to add more organisms as the supernatant fluid became clear. The greater the tendency to agglutinate, the larger the number of organisms necessary for complete absorption. The serum being absorbed had to be kept at ice-box temperature because of the well-known tendency of diluted serum to deteriorate at room or incubator temperature. A control of diluted serum which had been incubated under the same conditions as the test serum was necessary to determine whether a drop in titre had occurred, and a control of the serum to be tested saturated with homologous organisms indicated the completeness of the absorption.

WIDAL REACTIONS.

With the exception of a few microscopic tests for comparative purposes, the macroscopic test was used exclusively. Material was usually received, as drops of dried blood on glass and metal slides, or on filter paper. A few Widals were made from clear serum obtained in Wright's capsules. A dilution of 1-25 and 1-50 was made in saline and centrifuged when necessary. It was noticed that fresh serum drawn from the clot and used within twenty-four or forty-eight hours gave a positive agglutination with a larger number of organisms than did the Widals run with dried blood. When dried blood was used a precipitate was usually present in the tubes, giving a positive reaction. This might be due to the presence of hemoglobin, foreign substances on the metal slides or paper, some change in

reaction or biochemical change. No precipitation was noted in Widals where clear serum was used nor in the agglutination tests made with rabbit serum. An equal amount of an emulsion of organisms was added to the serum dilution, doubling the original dilution. All Widals were set up using strains Nos. 1, 2, 3, 10 and 12. The Rawlings strain, No. 12, was selected because it is widely used in the preparation of vaccines and for Widals. Nos. 1, 2 and 3 were used because of certain serological irregularities which they exhibited, and No. 10 because it was an organism giving a clear adherent agglutination with most sera used.

RESULTS.

Table III and IV give a summary of the agglutination reactions with immune sera. Table III shows that the same serum gave varying titres when different strains of *B. typhosus* were used as antigen. Table IV shows these differences even more clearly. From these results we have placed the organisms tentatively into three groups. Group I was made up of eleven organisms, group II of twelve organisms; group III of two organisms. Group I serum agglutinated all other organisms in this group in dilutions practically as high as it agglutinated the homologous organism. Group I serum also agglutinated group II organisms, but in lower dilutions. Conversely, the group I organisms are agglutinated by group II serum, but in lower dilutions than were the group II organisms. These two groups were closely related and interagglutinated to the degree indicated in the table. Groups I and II serum gave slight or no agglutination

TABLE III.—Quantitative variations in agglutinations with commercial sera.

NUMBER.	Sera used.					
	Parke-Davis.		Wichita.		University of Chicago.	
	Titre.	Reaction.	Titre.	Reaction.	Titre.	Reaction.
1	1–50	—	1–50	.....	1–8,000	4+
2	1–10,000	3+	1–50	1+	1–10,000	4+
4	1–1,000	4+	1–400	4+	1–2,000	4+
6	1–2,000	4+	1–400	.....	1–4,000	4+
50	1–50	—	1–50	.....	1–50	.....
51	1–50	—	1–50	.....	1–50	.....
52	1–50	.....	1–50	.....	1–50	.....
20	1–4,000	4+	.....	.....	1–8,000	4+

with group III organisms. Group III consisted of two strains, Nos. 2 and 3, which interagglutinated at 1-12,000, but this high-titred serum agglutinated members of groups I and II in low dilutions or not at all. The results of agglutination tests using the New York horse serum indicated that the same antigenic differences were present, but the titre of the serum was higher. To illustrate: No. 12, Rawlings', was completely agglutinated to 1-80,000, and No. 1 to 1-5,000.

It will be seen by inspection of Table V that the absorption tests showed a closer relationship between groups I and II than did the agglutination tests, strain No. 1 being somewhat more irregular than the other strains. The absorption tests showed a more striking difference between the two organisms in group III and the other groups. The antigenic differences shown by these organisms could not be correlated with their age as with Hooker's (1916) organisms nor with cultural differences as with Weiss' (1917). The antigenic differences between the strains shown by the use of immune serum were also apparent from the results of the Widal tests. If anything, these antigenic differences between the strains were exaggerated.

Table VI gives the results of the Widal tests. From this table it is readily seen that different organisms with the same sera set up at the same time showed marked differences in agglutinability. This may be due to the different agglutinogenic properties inherent in the organisms themselves. Such marked differences probably would not be noted had absorption tests been possible. It is recognized that these twenty positive Widals are too few to provide a basis for accurate conclusions. It seems highly probable that the dried blood method exaggerates the antigenic differences between the organisms, changing what is probably a quantitative into an apparently qualitative difference. The low percentage of positives with Nos. 2 and 3 might be expected from the results given in the absorption tests using immune serum. No. 10, on the other hand, gave a very low percentage of negatives. Those read as partial agglutination would, in clinical work, be called positive. The tubes read as positive gave complete clearing of the supernatant fluid; those read as partial agglutination showed unmistakable agglutination, but with some cloudiness of the supernatant fluid. No. 10, therefore, gave ninety-three per cent positive. No. 12, while giving the highest percentage of complete agglutinations, gave only ninety per cent positive when partial agglutinations are included. It seems probable in view of the results obtained that it might be worth while to use more than one

TABLE IV.—Agglutination reactions with immune sera.

STRAIN.	Serum.													
	1		4		7		20		12-Lederle.		2		3	
	Titre.	Reaction.	Titre.	Reaction.	Titre.	Reaction.	Titre.	Reaction.	Titre.	Reaction.	Titre.	Reaction.	Titre.	Reaction.
1	1–2000	.....	1–1000	.....	1–500	3+	1–2000	4+	1–2000	3+	1–100	4+	1–100	+
4	1–2000	.....	1–1000	.....	1–500	4+	1–4000	3+	1–2000	4+	1–100	+	1–100	±
6	1–2000	.....	1–500	.....	1–500	5+	1–2000	3+	1–5000	3+	1–100	+	1–100	±
7	1–1000	.....	1–1000	.....	1–3000	4+	1–3000	3+	1–8000	3+	1–100	2+	1–100	3+
8	1–200	.....	1–500	.....	1–3000	4+	1–2000	3+	1–2000	3+	.....	.....	.....	.....
20	1–1000	.....	1–1000	.....	1–500	3+	1–3000	4+	1–2000	3+	.....	.....	.....	.....
21	1–1000	.....	1–500	.....	1–2000	3+	1–2000	3+	1–2000	3+	.....	.....	.....	.....
23	1–200	.....	1–500	.....	1–1000	3+	1–1000	3+	1–8000	3+	.....	.....	1–200	3+
24	1–1000	.....	1–500	.....	1–3000	3+	1–3000	4+	1–8000	4+	.....	.....	1–100	2+
25	1–50	.....	1–1000	.....	1–1000	3+	1–3000	4+	1–5000	3+	.....	.....	1–200	3+
26	1–500	.....	1–2000	.....	1–3000	4+	1–3000	4+	.....	.....	.....	.....	.....	.....
27	.....	.....	.....	.....	1–1000	4+	.....	.....	1–1000	4+	.....	.....	.....	.....
28	.....	.....	.....	.....	1–1000	4+	1–1000	4+	1–5000	4+	.....	.....	1–50	3+
29	.....	.....	.....	.....	1–500	4+	1–1000	4+	1–5000	.....	.....	.....	1–50	2+
11	1–200	.....	1–500	.....	1–1000	3+	1–500	3+	1–8000	4+	1–200	4+	1–100	4+
9	1–2000	.....	1–2000	.....	1–2000	3+	1–3000	3+	1–5000	3+	1–1000	3+	1–1000	3+
12	1–1000	.....	1–2000	.....	1–2000	4+	1–2000	4+	1–8000	3+	.....	.....	.....	.....
10	1–1000	.....	1–2000	.....	1–500	3+	1–2000	3+	1–5000	3+	1–200	3+	1–1000	3+
13	1–1000	.....	1–1000	.....	1–200	3+	1–2000	3+	1–5000	4+	1–200	4+	1–200	3+
14	1–1000	.....	1–2000	.....	1–1000	3+	1–1000	3+	1–2000	3+	1–200	3+	1–1000	3+
15	1–1000	.....	1–500	.....	1–2000	3+	1–2000	3+	1–500	3+	1–100	3+	.....	.....
16	1–1000	.....	1–5000	.....	1–5000	3+	1–3000	3+	1–5000	4+	1–100	2+	1–100	4+
17	1–1000	.....	1–5000	.....	1–1000	3+	1–300	3+	1–5000	3+	1–100	2+	.....	.....
19	1–1000	.....	1–1000	.....	1–1000	3+	1–1000	3+	1–5000	4+	1–100	2+	1–100	±
30	.....	.....	.....	.....	1–1000	4+	1–1000	4+	1–8000	4+	1–50	3+	1–50	3+
31	.....	.....	.....	.....	1–100	4+	1–1000	4+	1–8000	4+	1–50	±	1–50	2+
32	.....	.....	.....	.....	.....	.....	1–100	4+	1–5000	3+	.....	.....	1–50	3+
2	1–200	.....	1–500	.....	±	.....	±	.....	1–50	3+	1–15000	4+	1–12000	3+
3	1–200	.....	.....	.....	+	.....	±	.....	1–50	3+	1–15000	3+	1–12000	4+

TABLE V.—Absorption tests with immune sera.

Absorbing antigen.	Sera used.									
	1	12	9	2	3	27	7	13	20	8
1	+	±	±	±	—	±	±	+	±	±
4	+	+	+	—	—	+	+	+	+	+
6	±	+	±	—	—	—	+	+	.....	+
7	±	+	+	—	±	+	+	+	+	+
8	±	+	+	—	—	—	+	+	+	+
20	±	±	+	.....	—	+	+	+	+	+
21	+	+	±	—	—	+	+	+	+	+
23	+	±	+	±	±	+	+	+	+	+
24	+	+	+	—	—	+	+	+	+	+
25	+	±	+	—	—	+	+	+	+	+
26	+	+	±	—	—	+	+	+	+	+
27	+	—	+	—	—	+	.....	+	+	+
9	+	+	+	—	—	+	+	+	+	.....
10	+	+	+	—	—	+	—	±	+	±
11	±	+	+	—	—	+	—	+	—	—
12	±	+	+	±	—	±	—	+	+	+
13	+	+	+	—	—	±	±	+	±	+
14	+	+	±	+	+	±	.....	+	+	+
15	±	—	+	—	.....	±	+	+	+	+
16	+	+	+	—	.....	+	+	+	+	+
17	+	±	+	±	—	+	+	+	—	+
19	+	+	+	—	.....	+	+	+	+	+
2	—	±	—	+	+	—	—	—	—	—
3	—	±	—	+	+	—	—	—	—	—

+ Absorption complete.
± Absorption incomplete but reduction of titre.
— No absorption.

TABLE VI.—Widal reactions.

Organisms.	Sera.																			Percentages.			
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	+	±	—
1	—	—	—	—	+	—	—	....	+	+	±	—	—	+	+	+	+	+	—	±	42	10	47
2	—	±	+	—	—	±	±	—	+	—	—	±	—	+	—	+	±	—	—	—	20	25	55
3	—	....	+	±	±	±	+	—	+	....	—	—	+	+	±	+	±	—	—	—	33	27	38
10	+	....	+	+	+	+	+	....	+	....	±	±	±	±	—	±	+	+	+	+	70	23	6
12	+	+	+	+	+	+	—	+	+	+	+	—	±	+	+	±	±	+	+	+	75	15	10

+ Clear-cut, complete agglutination.
± Partial agglutination.
— Negative agglutination.

strain of *B. typhosus* in running Widals and to select easily agglutinable strains such as the Mt. Sinai strain (No. 10) or the Rawlings strain (No. 12).

DISCUSSION.

These antigenic differences shown by agglutination, absorption and Widal tests are abundantly confirmed by an examination of the literature. Resistance to agglutination of some strains of *B. typhosus* recently isolated from the blood or feces is well known, and these strains usually become agglutinable, after growth on artificial culture media. We are not concerned with such strains in this case. Sheller (1908) and Burri and Dugeli (1909) report the isolation of organisms from convalescent and healthy carriers culturally identical with *B. typhosus* but which were inagglutinable. Since, at this time, some confusion existed regarding the cultural characteristics of *B. typhosus* their results cannot be accepted without question.

Raubitschek (1913), using organisms isolated from the different organs of a fatal case of typhoid, reports remarkable differences in agglutinability in these organisms which under the conditions of the test would appear to be different individuals of one strain. High-titred serum was prepared by immunizing rabbits with an old laboratory strain. It might be suggested that this strain was one resembling our No. 2 or No. 3 which gave a serum agglutinating few organisms other than the homologous organism. Teague and Torrey (1907) and Taskin, cited by Garbat (1916) report the occurrence of irregular strains of *B. typhosus*. Durham (1901) observed antigenic differences, but did not attempt to group his strains on this basis. Garbat (1916) considered the antigenic differences of enough importance that he used seven or eight strains in the preparation of his antigen for complement-fixation work. Mock (1919) reports atypical serological and cultural reactions in organisms isolated from typhoid patients who had been vaccinated about one year previous to the attack. Myers (1920) reports the isolation of an atypical strain from the blood stream of a vaccinated individual. Hooker (1916) worked out two groups and one subgroup among typhoid organisms by the use of the complement-fixation test. Group I showed cross-fixation with all antigens, group II with each other but not with group I, group I-A gave irregular results. He finds that the Rawlings strain seems to possess less antigenic complexity than does any other organism. It should, therefore, have less value as an immunizing strain. His absorption tests showed that groups I and II

were quite distinct. Weiss (1917) was able to divide his organisms into two well-marked groups, and a third group containing an inagglutinable, rhamnose-fermenting organism. These antigenic differences were correlated with irregularities in xylose fermentation.

It may be seen from Tables III and IV that there exist undoubted antigenic differences in the strains tested. The differences seemed to be quantitative rather than qualitative, since most sera gave agglutination with all organisms in low dilutions.

The antigenic differences became more apparent and seemed to possess more significance in the Widal tests. Table VI indicates the degree to which the organisms differed.

The serological reactions here recorded as evidenced by agglutination, absorption and Widal tests might have an important bearing on the following points:

(1) Sources of error in the Widal run with dried blood.
(2) The occurrence of negative Widals in clinical cases of typhoid fever.
(3) The occurrence of typhoid fever in vaccinated cases.
(4) The advisability of using a polyvalent vaccine.
(5) The correlation between cultural and antigenic differences.

My results indicate that a Widal using dried blood gives a weaker reaction than one run with clear serum. This tends to exaggerate the antigenic differences between the organisms. In Widal tests, an easily agglutinable organism should be chosen, since the less agglutinable types may be entirely negative, even though the same serum gives a complete agglutination with another strain. These results may be due to a decrease in the activity of the agglutinins from drying or to the presence of harmful substances on the material on which the blood is dried.

Stober (1904) and Mock (1919) report irregularities in Widal reactions from clinical cases of typhoid while Robinson (1915), on the other hand, reports no variability in one hundred Widals using the Worcester and Rawlings strains. It is very possible that when using two easily agglutinable strains such as the Rawlings and Mt. Sinai strains no significant differences could be observed. The antigenic peculiarities discussed here might account for the occurrence of negative Widals in typhoid fever. If the strain infecting the patient were not antigenically similar to the strain used for the Widal it is conceivable that an appreciable amount of agglutinin would not be present in the patient's blood for the strain used in the laboratory. Conversely, if the laboratory used one strain only, and

that one happened to be one of the less easily agglutinable strains it is possible that the patient's blood might not agglutinate it. The antigenic differences demonstrated between the Rawlings strain and No. 2 bear out this contention. Hawn, Hopkins and Meader (1918) report forty per cent of positive Widals in a small epidemic among American troops. The source of the organisms used for the Widals is not given. From Table VI it will be seen that the number of positives with Nos. 1, 2 and 3 range from twenty to forty per cent, while with Nos. 12 and 13 they range from seventy to seventy-five per cent. Where the percentage of positive Widals is low, as in Hawn's (1918) cases, the organism infecting the patient or used by the laboratory might be of some importance. It is always possible that a negative Widal may be due to a paratyphoid infection, so that it seems wise to use *B. paratyphosus A* and *B* as well as at least one easily agglutinable strain of *B. typhosus*. Manteufel (1921) would go even farther and set up each Widal with *B. suipestifer* and *B. enteritidis* as well as *B. paratyphosus A* and *B*, and *B typhosus*, basing his recommendation on the occurrence of all these organisms in cases and carriers.

A number of cases of typhoid occurring in vaccinated individuals may be found in the literature. There were numerous cases of "so-called" typhoid reported in the German army and in the French army prior to 1915, when the vaccine containing *B. typhosus*, *B. paratyphosus A* and *B* was first used. It is reasonable to assume, therefore, that many of these cases were really paratyphoid infections. There were a few undoubted cases of typhoid in the American army among vaccinated soldiers. Brown, Palfrey and Hart (1919) report eighteen cases of clinical typhoid with isolation of the organisms from the blood stream in the majority of cases. These men had been vaccinated within the year with the army vaccine.

Hawn, Hopkins and Meader (1918) report the occurrence of thirty-eight cases of typhoid among vaccinated men, and suggest three possibilities as the cause: First, defective vaccination; second, infection with a different strain; third, overwhelming infection.

Goodall (1918) reports a number of cases among Belgians, French and English. Here, there seems to be some doubt whether the cases were not paratyphoid infections. Protection against paratyphoid was imperfect at this time. Vaughn (1919) says that, "It is possible that in so far as vaccination has failed it is due to the disease being caused by other members of the typhoid group, . . . which in all probability is much larger than we now appreciate." Mock

(1919) reports the occurrence of forty-five cases of typhoid and paratyphoid in individuals who had been vaccinated about one year previous to the attack. Trowbridge (1915) reports the occurrence of a typhoid epidemic among vaccinated persons in an institution, where the original source of infection came from the milk supply which was infected by a vaccinated worker with a mild case of typhoid. It is realized that in such an epidemic the dosage may have been sufficient to overcome the immunity acquired from vaccination. Wade and McDaniels (1915) report the occurrence of an epidemic among vaccinated individuals. Here there seemed to be an interesting correlation between the negative Widals given after vaccination and the susceptibility of these persons to typhoid.

Hooker (1916) and Weiss (1917) conclude from their experiments that a vaccine made from several strains of *B. typhosus* would be more efficient than one made from a single strain. Brown (1919) also suggests this possibility. The results of these observers and the cases of typhoid in vaccinated individuals as cited above, together with my findings would suggest that it might be well to consider the use of a vaccine made from several strains. Teague (1920), on the other hand, rather arbitrarily dismisses the necessity of using more than one strain in vaccines on the grounds that the antigenic differences noted are not permanent. The antigenic differences between my strains have persisted for three years.

An examination of the results reported in experiments I and II showed that there was very little correlation between cultural and antigenic characters. Cultures Nos. 12, 21 and 40, which were irregular in xylose fermentation were all typical in agglutination reactions and belonged to group II, which gave good cross-agglutination with other strains. This is not in agreement with the observations of Weiss (1917). Strain No. 1 gave an unusually rapid reversion to alkaline in litmus milk and was somewhat less agglutinable than many of the other strains. Strains 2 and 3 were typical in their cultural reactions but were less agglutinable than most of my other strains. Strain 5 was inagglutinable and fermented rhamnose but not xylose. It, therefore, differed from the other strains in cultural and antigenic properties. The identity of strain 5 remains unsettled. It gave no agglutination with any of the antityphoid sera used, nor did it reduce the titre in absorption tests. Conversely, the serum produced by the inoculation of this organism into a rabbit did not agglutinate any of the other organisms used, nor did any of the typical typhoid strains reduce the

titre of strain 5 serum. This organism did not agglutinate with stock serum for *B. paratyphosus A* or *B*, nor with a *B. sanguinarium* or *B. pullorum* antiserum. After three years this strain remained inagglutinable in antityphoid serum. Weiss (1917) reported on an entirely inagglutinable strain of this type. Sheller (1908) has reported on an inagglutinable strain. Myers (1920) reported the isolation of a rhamnose-positive *B. typhosus* which was also atypical in its serological reactions. Krumwiede (1918) feels, in view of the apparent serological relations between *B. sanguinarium* and *B. pullorum* and *B. typhosus*, that the fermentation of rhamnose is a more fundamental character than serological reactions. Krumwiede using this fermentation as the deciding factor between the typhoid and paratyphoid group would place the Weiss organism and strain 5 in the paratyphoid group.

CONCLUSIONS.

In summing up the work done the following conclusions were reached:

1. Culturally, the strains of *B. typhosus* used differed slightly from each other, the reaction being most variable in dextrin, xylose, salicin and litmus milk. These variations could not be correlated with the age of the culture nor the source.

2. Cross-agglutination and absorption tests established the existence of quantitative antigenic differences between the strains used. It appeared that the conflicting opinions as to whether there are antigenic differences in the typhoid group may be due to the fact that qualitative rather than quantitative differences have been emphasized.

3. There was no significant correlation between the cultural and antigenic peculiarities demonstrated.

4. The antigenic differences exhibited could not be correlated with the age of the organisms.

5. There was a marked difference in the agglutination of different strains of *B. typhosus* by the serum used in Widals and it would be advisable to set up each Widal with more than one strain, selecting strains which were known to give a high percentage of positive reactions.

6. For the Widal the use of fresh serum drawn from the clot was much more satisfactory than the use of dried blood.

7. An organism in some respects resembling *B. typhosus* but exhibiting cultural and antigenic irregularities was described.

PART II.

METABOLIC STUDIES.

PURPOSE.

The purpose of the second part of my investigations may be summarized as follows:

1. To study the relation of hydrogen-ion concentration to growth, vitality, and fermentation reactions of *B. typhosus*.

2. To study the sugar consumption of *B. typhosus* and correlate this with other factors such as growth rates, titratable acidity and change in hydrogen-ion concentration.

3. To tabulate and compare the characters of all of the strains of *B. typhosus* studied and note any correlation between antigenic variations and other characteristics.

METHODS AND TECHNIQUE.

ELECTROMETRIC. For the measurement of hydrogen-ion concentrations both electrometric and colorimetric methods were used.

The electrometric method was used for checking all buffer solutions and in many of the other determinations where a check on the colorimetric method was desirable or where especial accuracy was required. A Leeds and Northrup potentiometer with a Clark (1922) bubbling electrode was used. In the following tables electrometric determinations may be recognized, as they are expressed in four figures, while no attempt was made to read colorimetric determinations closer than .1 pH.

For the colorimetric determinations buffer solutions, made according to the directions given by Clark and Lubs (1917*a*), were used. These buffer solutions were checked by the electrometric method and corrected when necessary.

The phenol-sulphon-phthalein indicators recommended by Clark and Lubs (1917) were used. A comparator such as the one described by Hurwitz *et al.* (1916) was found satisfactory. The tubes containing the buffer solutions, with indicator added, were superimposed on tubes containing the medium to be tested, diluted 1-4, but without indicator. This compensates for the turbidity and color of the medium to be tested. Clark and Lubs (1917) state that a medium may be diluted four to five times without an appreciable change in hydrogen-ion concentration. This dilution reduces the errors due to turbidity and color. One cubic centimeter of the medium to be tested was diluted to 5 cc. with freshly distilled water

and two drops of indicator added. This tube was placed between the two buffer solutions and the color compared.

Clark and Lubs (1917), Foster (1921 *a* and *b*), Avery and Cullen (1919*a*), Dernby and Avery (1918) and others have reported that accurate readings may be made within 0.05 to 0.1 pH if the proper precautions are observed. This also has been my experience.

CULTURE MEDIA.

For viability and purity tests eosin-methylene-blue lactose agar plates, Holt-Harris and Teague (1916) were used. For the determination of growth rates, meat-infusion broth agar adjusted to pH 7.2 was found to be satisfactory. For the metabolism work meat-infusion broth was used, to which was added buffer solutions as indicated in the experiments and the percentage of carbohydrates desired.

EXPERIMENTAL.

The studies included in the following experimental work comprise a study of:

1. Effect of hydrogen-ion concentration on *B. typhosus*.
   (*a*) The range of growth of *B. typhosus*.
   (*b*) The change of hydrogen-ion concentration in plain broth.
   (*c*) The final or limiting hydrogen-ion concentration in broth containing various fermentable carbohydrates.
   (*d*) The viability of *B. typhosus* in broth containing a fermentable carbohydrate.
   (*e*) The influence of varying factors on the final hydrogen-ion concentration in dextrose broth.
2. Dextrose metabolism and correlated factors.
   (*a*) Utilization of glucose and influence of buffers.
   (*b*) Rate of acid formation.
   (*c*) Growth rates.
   (*d*) Rate and relation of the utilization of dextrose to final hydrogen-ion concentration and titratable acidity, inhibition and death.

QUALITATIVE TESTS.—EFFECT OF HYDROGEN-ION CONCENTRATION ON B. TYPHOSUS.

(*a*) *Range of Growth for B. typhosus.*

MEDIA. Meat infusion broth was titrated, using phenolphthalein, and divided into nine lots of 150 cc. each in hard-glass flasks. A calculated amount of acid, N/1 HCl, and alkali, N/1 NaOH, as the case might be, was added to bring the reaction to the approximate hydrogen-ion concentration desired. Each lot was then tested colorimetrically with a suitable indicator and further adjustment made

TABLE VII.—Range of growth of *B. typhosus* in plain broth expressed in logs.

ORGANISM.	Original number of organisms.	pH 4.564.	pH 5.001.	pH 5.764.	pH 6.576.	pH 7.134.	pH 7.658.	pH 8.115.	pH 8.402.	pH 9.636.
12	5.9212	sterile	4.5315	8.8949	9.0000	9.0086	8.6021	7.8388	7.3324	7.3617
5	6.5717	sterile	sterile	7.7242	9.0569	8.9345	9.1461	9.1461	8.8865	8.3010
1	4.9542	sterile	sterile	7.4771	7.9085	8.6021	8.2041	8.3010	6.7782	sterile
2	5.6180	sterile	4.5315	7.9243	7.7243	8.8513	8.7782	7.8865	6.4683	sterile
9	5.8651	sterile	sterile	8.6857	8.9058	9.0086	8.0453	7.8386	7.3324	sterile
4	5.7924	sterile	sterile	7.4771	7.9085	7.9542	8.2041	7.9581	6.7782	sterile
13	5.7709	sterile	sterile	7.9959	7.9325	8.9494	8.8808	7.8195	6.4624	sterile

if necessary. The media was then sterilized and electrometric determination made just before inoculation. Each flask was inoculated with 0.5 cc. of an eighteen-hour broth culture. A number of dilutions in sterile water blanks were made immediately and plated. The average number of colonies from all the flasks was taken as shown in Table VII. It was found necessary to make the plates immediately, since a decrease of organisms was observed in the more acid flasks shortly after inoculation. After eighteen hours in the incubator 5 cc. were removed from each flask, the proper dilutions made and plated. All plates were made in duplicate or triplicate. A sample was also removed for hydrogen-ion determination. In all cases where growth had occurred there was a slight increase in alkalinity from 0.1 to 0.3 pH.

Table VII shows the range of growth of organisms which represent typical examples taken from many determinations. It is evident in every case that no growth was present after eighteen hours at pH 4.5, that is to say, this degree of acid had sterilized the medium. At pH 5.0 only two organisms, No. 12 and No. 2, were viable, and the number of organisms was less than the original. The maximum growth took place between pH 6.0 and pH 7.5 with the exception of No. 5, where growth was vigorous, but decreasing at pH 9.6. With the exception of Nos. 12 and 5 all were sterile at pH 9.6.

Fennel and Fisher (1919) found that *B. typhosus* grew on nutrient agar from pH 4.0 to pH 9.6 with an optimum of pH 6.2 to pH 7.2. Dernby (1921) gives the optimum as pH 6.8 to 7.2. Schoenholz and Meyer (1921), using a heavily buffered meat infusion broth, found that growth was slight or none at pH 4.3 and pH 4.8; slight at pH 9.0, and most vigorous at pH 6.6 and pH 6.8. They also make the statement that although *B. typhosus* grows better in a slightly acid environment, it is more tolerant of alkali than of acid. This was found to be the case in my experiments also. These same authors found that in a medium containing bile salts growth was greatly inhibited at pH 8.2. Wright (1917) observed that the growth of *B. typhosus* was light at pH 4.5-5.0, heaviest at pH 6.0-6.5 and declined sharply at pH 7.5-8.0. Dernby and Näslund (1923) give the limits for growth for *B. typhosus* as pH 4.6-8.0 with an optimum of pH 6.5-7.2. Hall and Fraser (1922) give the limits of growth at pH 4.0-8.6, using a peptone solution made from pure casein.

My results are comparable to those of Schoenholz and Meyer (1921), Wright (1917), Dernby and Näslund (1923), and Hall

and Fraser (1922). Fennel and Fisher's (1919) results suggest either that the organisms grew over a wider range of hydrogen-ion concentration in solid media than in liquid media, or that these authors were using an organism similar to No. 12 or No. 5, which exhibited unusually vigorous growth over a wide range. With the exception of strains 12, 2 and 5 the limits of growth for my organisms were pH 5.5-8.5. The optimum was from pH 6.0 to 7.5 and the different strains did not differ markedly from one another. The limits of growth for No. 2 was from pH 5.0 to pH 8.5, for No. 12 from pH 5.0 to pH 9.0, and No. 5 from pH 5.5 to pH 9.6. It is interesting to observe that antigenically these organisms were quite distinct, No. 2 belonging to group III and interagglutinating well only with No. 3. No. 12, the Rawlings strain, is a member of the larger and more homogeneous group II, and No. 5 is the inagglutinable strain.

From my results and those of the authors cited, it is evident that *B. typhosus* is unable to withstand pH 4.5 for twenty-four hours, and is able to multiply feebly at pH 5.0 on the acid side. It multiplies fairly well at pH 8.0, but from that point on growth is increasingly inhibited, until at pH 9.0 the great majority of strains are sterile after twenty-four hours.

The optimum range is wider in my series than in that of most authors with the exception of Dernby and Näslund (1923). However, the end point as well as the optimum zone depends upon the amount inoculated, the age of the inoculum and the composition of the medium. There was no apparent relation between the length of time the cultures had been isolated and the optimum zone, the most recently isolated culture showing no marked difference from those which had been growing on culture media for thirty-three years. This is not in accord with the findings of Schoenholz and Meyer (1921). Such a range of optimum growth gives a fairly wide latitude in the selection and adjustment of culture media. This is in marked contrast to an organism like the pneumococcus where the range of optimum growth occurs between pH 7.4-7.8.

It may be pointed out, however, that the reaction of Endo's medium, pH 7.8-8.4, would inhibit the growth of *B. typhosus* to a certain extent.

(*b*) *Change of Hydrogen-ion Concentration in Plain Broth.*

It is difficult to understand why the statement is so frequently made that the growth of organisms in plain broth produces no change in reaction. Russel *et al.*, cited by Esty (1921), and Dernby (1923), state that there is no change in the reaction of culture media produced by *B. typhosus* if the medium is free of dextrose. Hajes (1922) states that there is no change in hydrogen-ion concentration in plain broth medium using a variety of organisms. D'Aunoy (1923), using *B. pestis* and the colorimetric method, found no change in hydrogen-ion concentration in plain broth medium. In contrast to the above statements, are those of Peré (1892), Hulton-Fraenkel (1919*a*), and others, that an increased alkalinity is present after several day's incubation in plain broth or in broth containing so small an amount of carbohydrate that it may be completely used. Cobbett (1897), Bunker (1919), Hartley (1922), and others, have shown that a distinct alkaline reversion takes place in diphtheria cultures in a medium containing a small amount of dextrose, and that this alkaline reversion is synchronous with toxin production.

Table VIII gives a summary of the titratable acidity and change in hydrogen-ion concentration in a highly buffered broth produced by twenty-five typical strains of *B. typhosus* and one atypical strain, No. 5. It will be seen that there was a slight increase in titratable acid during the first twenty-four hours, accompanied by a small increase in hydrogen-ions which was within the limits of error for the colorimetric test. In forty-eight and seventy-two hours there was a progressive decrease in titratable acidity and decrease in free hydrogen-ions.

With the exception of No. 5, the changes in hydrogen-ion concentration in plain meat infusion broth were remarkably constant for the different strains. Strain 5 showed a more marked alkaline production than did the others. This strain also differed antigenically. This progressive alkalinity was observed in all of our cultures under the following conditions: First, meat-infusion broth to which no fermentable carbohydrate was added; second, broth to which such small amounts of carbohydrate was added that the organisms were able to ferment it completely without marked inhibition of growth; third, broth to which a carbohydrate was added which was not attacked by *B. typhosus*, such as saccharose.

The slight increase in titratable acidity was probably due to the fermentation of the small amount of dextrose present in meat in-

fusion broth. Kendall (1913) suggests that this increased acid might also be due to the fermentation of the so-called carbohydrate moiety of some of the proteins in the peptone. He attributes the

TABLE VIII.—Titratable acidity and hydrogen-ion concentration in buffered meat-infusion broth to which no dextrose was added. Summary of results with twenty-five organisms and strain No. 5 listed separately.

ORGANISM.	Original pH.	Titratable acidity.	pH 24 hours.	Titratable acidity 24 hours.	pH 48 hours.	Titratable acidity 48 hours.	pH 72 hours.	Titratable acidity 72 hours.
Summary.....	7.8	4.47	7.7	4.95	8.03	4.18	8.15	2.42
No. 5.....	7.8	4.48	7.6	6.02	8.2	4.80	8.6	1.42

progressive alkalinity to an increase in alkaline products, chiefly ammonia from the proteins. The organisms deamidize the proteins prior to the intracellular use of the nitrogen-free portion for energy.

It seems likely that those persons who did not observe this alkalinity failed to do so because of the lack of suitable indicators or the failure to make observations over a sufficient period of time.

(c) *The Final Hydrogen-ion Concentration in Broth Containing Various Fermentable Carbohydrates.*

This experiment may be divided into three parts: First, the final or limiting hydrogen-ion concentration in 1.0 per cent carbohydrate broth; second, the final hydrogen-ion concentration in 2.0 per cent carbohydrate broth; third, the effect on fermentation of growth in the presence of a carbohydrate which is fermented with difficulty.

In every case the desired amount of sterile carbohydrate was added to sterile meat-infusion broth adjusted to the hydrogen-ion concentration indicated in the tables. The hydrogen-ion concentration was determined in some cases by electrometric, in others by colorimetric methods and the results were checked many times. Dextrose, mannite, maltose, xylose and dextrin were used, as it was felt that these carbohydrates were representative of the different carbohydrates fermented by *B. typhosus*.

TABLE IX.—Hydrogen-ion concentration and day of death in 1 per cent dextrose broth.

ORGANISM.	Original pH.	pH 24 hours.	pH 48 hours.	pH 72 hours.	Growth, 72 hours.
1	7.0	5.1	5.2	5.2	0
2	7.2	5.1	5.2	5.2	0
3		5.1	5.1	5.0	0
4	7.0	5.0	4.9	5.2	+
5	7.0	5.2	5.5	5.5	+
9	7.0	5.1	5.1	5.1	0
12	7.0	5.2	5.2	5.2	0
13	7.0	5.2	5.2	5.1	0
14	7.1	5.0	5.0	5.1	0
15	7.2	4.9	5.0	5.0	0
17	7.1	4.9	5.1	5.2	0
21	7.2	4.9	4.9	4.9	+
23	7.1	5.0	4.9	4.9	+
24	7.2	5.0	5.1	5.0	0
25	7.0	5.0	5.1	5.2	0
26	7.0	4.9	5.0	5.1	0
27	7.0	5.1	5.1	5.2	0
28	7.0	5.1	5.1	5.1	+
29	7.1	5.1	5.1	5.0	0
30	7.2	5.1	5.1	5.1	0
32	6.8	5.0	5.0	5.1	+
39	6.8	5.1	5.1	5.2	0
40	7.2	5.0	5.0	5.1	0
44	6.8	5.1	5.1	5.2	0
45		5.1	5.1	5.1	0

Table IX gives the hydrogen-ion concentration in 1 per cent dextrose broth. It will be seen that in every case the final hydrogen-ion concentration was reached in twenty-four hours. The final hydrogen-ion concentration reached in most cases was pH 5.1 and the greatest was pH 4.9. In a few cases there was a slight alkaline reversion after seventy-two hours, or one week, amounting to 0.1-0.3 pH. Six organisms showed growth after seventy-two hours, and one only, No. 5, after one week.

TABLE X.—Hydrogen-ion concentration and day of death in 1 per cent mannite broth.

ORGANISMS.	Original pH.	pH 24 hours.	pH 48 hours.	pH 72 hours.	Growth, 72 hours.
1	7.2	5.3	5.3	5.4	0
2	7.2	5.2	5.3	5.4	0
3	7.2	5.2	5.2	5.3	+
4	7.2	5.2	5.2	5.3	+
5	7.2	5.6	5.8	5.8	0
9	7.2	5.3	5.3	5.3	0
12	7.4	5.1	5.0	5.2	0
13	7.4	5.1	5.2	5.5	+
14	7.4	5.2	5.2	5.3	+
17	7.4	5.2	5.1	5.1	0
21	7.4	5.1	5.1	5.3	0
23	7.4	5.3	5.1	5.2	0
24	7.4	5.2	5.1	5.4	0
25	7.4	5.2	5.2	5.0	+
26	7.4	5.2	5.3	5.3	0
27	7.4	5.1	5.1	5.3	0
28	7.4	5.1	5.2	5.5	0
29	7.4	5.1	5.1	5.3	0
30	7.4	5.1	5.2	5.4	0
32	7.4	5.2	5.2	5.4	0
39	7.4	5.1	5.1	5.2	+
40	7.4	5.2	5.1	5.2	0
44	7.4	5.1	5.2	5.3	0
45	7.4	5.1	5.1	5.1	0

Table X gives the hydrogen-ion concentration in 1 per cent mannite broth. The majority of strains reached a pH of 5.1 but none were more acid than this. With four exceptions, this final hydrogen-ion concentration was reached in twenty-four hours. All showed slight alkaline reversion after one week. Six out of twenty-five strains were not viable in seventy-two hours.

TABLE XI.—Hydrogen-ion concentration and day of death in 1 per cent maltose broth.

ORGANISM.	Original pH.	pH 24 hours.	pH 48 hours.	pH 72 hours.	Growth, 72 hours.	1 Week.	Growth, 2 weeks.
1.....	7.6	6.021	6.0	6.0	+ +	5.8	+ +
2.....	7.6	6.2	5.7	5.4	+ +	5.7	— —
3.....	7.6	6.021	5.528	5.782	+ +	5.444	+ +
4.....	7.6	5.6	6.0	.....	.....	6.0	— —
5.....	7.6	6.195	5.985	8.098	+ +	8.706	+ +
9.....	7.6	5.8	5.5	5.6	+ +	5.8	— —
12.....	7.6	6.021	5.545	5.579	+ +	5.511	— —
13.....	7.6	6.1	5.8	5.4	+ +	5.8	+ +
14.....	7.6	6.1	6.2	5.6	+ +	5.6	— —
17.....	7.6	6.8	6.2	5.6	+ +	5.6	— —
21.....	7.6	6.096	5.579	5.647	+ +	5.444	— —
23.....	7.6	6.1	6.1	5.4	+ +	5.7	+ +
24.....	7.6	6.1	6.1	5.3	+ +	5.8	+ +
25.....	7.6	6.1	5.7	5.7	+ +	5.7	+ +
26.....	7.6	6.1	5.6	5.4	+ +	5.8	—
27.....	7.6	6.2	5.8	5.4	+ +	5.8	+ +
28.....	7.6	6.3	5.5	5.4	+ +	6.4	+ +
29.....	7.6	6.2	5.8	5.4	+ +	5.8	+ +
30.....	7.6	6.1	5.7	5.5	+ +	5.8	— —
32.....	7.6	6.1	5.6	5.4	+ +	5.8	+ +
39.....	7.6	6.2	5.5	5.5	+ +	5.6	+ +
40.....	7.6	6.3	5.4	5.6	+ +	5.6	— —
44.....	7.6	5.9	5.8	5.7	+ +	5.8	—
45.....	7.6	5.9	6.0	5.5	+ +	5.8	—

Table XI gives the hydrogen-ion concentration in maltose broth. There is much greater variation in the final hydrogen-ion concentration reached and the length of time involved. The final hydrogen-ion concentration reached was from pH 5.3 to pH 6.1. It will be observed that most of the organisms reached their final hydrogen-ion concentration only after seventy-two hours incubation. The highest hydrogen-ion concentration reached in twenty-four hours was pH 5.6. After seventy-two hours' incubation, all organisms were living. After one week, twelve were living and thirteen were dead. Strain No. 5 differed from the other organisms in showing marked alkaline reversion after forty-eight hours. This was progressive in spite of the continued presence of maltose in the medium.

TABLE XII.—Hydrogen-ion concentration and day of death in 1 per cent xylose broth.

ORGANISM.	Original pH.	pH 24 hours.	pH 48 hours.	pH 72 hours.	Growth, 72 hours.	pH 1 week.	Growth, 1 week.
1.....	7.2	5.6	5.6	5.7	+	6.2	—
2.....	6.948	5.292	5.258	5.172	—	5.258	—
3.....	6.9	5.6	5.6	5.7	+	5.9	—
4.....	6.9	5.6	5.4	5.8	+	5.8	—
5.....	6.931	7.303	7.777	8.284	+	8.537	+
9.....	7.2	5.4	5.5	5.6	+	5.9	+
12.....	7.236	7.151	7.523	7.574	+	6.728	+ +
13.....	7.185	5.5	5.5	6.0	+	6.5	+ +
14.....	7.1	5.5	5.5	5.6	+	5.6	+
15.....	7.0	5.5	5.5	5.6	+	5.9	+
17.....	7.2	5.4	5.4	6.0	+	6.2	—
21.....	6.931	7.320	7.608	7.743	+	7.658	+
23.....	7.2	5.8	5.8	6.0	+	6.2	+
24.....	7.2	5.4	5.4	5.7	+	5.7	+
25.....	7.1	5.5	5.5	5.8	+	5.8	+
26.....	7.2	5.6	5.6	5.6	+	5.7	+
27.....	7.1	5.4	5.4	5.8	+	5.8	—
28.....	7.1	5.4	5.4	5.6	+	5.6	—
29.....	6.914	5.376	5.258	5.292	—	5.697	—
30.....	6.914	5.241	5.292	5.292	—	5.714	—
32.....	7.2	5.4	5.4	5.6	+	5.7	—
34.....	6.9	5.5	5.6	5.6	+	5.6	—
40.....	6.948	6.661	6.948	7.067	+	7.236	+
44.....	6.948	5.3	5.3	5.7	+	5.9	+
45.....	6.9	6.2	6.0	6.0	+	6.6	+

Table XII gives the final hydrogen-ion concentration and the day of death of *B. typhosus* in 1 per cent xylose broth. The lowest point, pH 5.1, was reached by only one organism and after seventy-two hours' incubation. The hydrogen-ion concentration reached by the majority was from pH 5.2 to 5.6. There was slight alkaline reversion in all tests, and all organisms were viable after seventy-two hours. Eleven organisms were not viable after one week. Three organisms, namely: Nos. 5, 21 and 40, did not ferment xylose at all during the period covered in the table. The broth became progressively alkalin as if there were no carbohydrate present. The Rawlings strain, No. 12, gave slight acid after one week. No. 21 produced slight acid

in twenty-four hours and then became progressively alkaline for about two weeks. At the end of three weeks it had reached pH 6.5. At the end of three weeks No. 12 and No. 40 had reached pH 5.5 and 5.8, respectively. Strain No. 5 produced a progressively alkaline reaction, giving pH 8.4 at the end of three weeks.

TABLE XIII.—Hydrogen-ion concentration and day of death in 1 per cent dextrin broth.

ORGANISM.	Original pH.	pH 24 hours.	pH 48 hours.	pH 72 hours.	Growth, 72 hours.	pH 1 week.	Growth, 1 week.
1.....	7.2	6.2	7.0	7.4	+	7.5	+
2.....	7.2	6.2	7.0	7.0	+	7.6	+
3.....	7.2	5.6	6.4	6.6	+	7.5	+
4.....	7.2	6.6	6.8	6.8	+	7.2	+
5.....	7.2	5.6	7.2	7.2	+	7.5	+
9.....	7.2	5.6	5.5	6.0	+	7.5	+
12.....	7.2	6.2	6.8	6.8	+	7.6	+
13.....	7.2	6.4	6.8	6.8	+	7.5	+
14.....	7.2	6.3	6.0	6.9	+	7.4	+
15.....	7.2	5.6	6.3	6.9	+	7.4	+
17.....	7.2	5.2	6.1	6.7	+	7.3	+
21.....	7.2	5.9	6.3	7.0	+	7.3	+
23.....	7.2	6.0	6.5	6.8	+	7.2	+
24.....	7.2	5.9	5.8	6.8	+	7.4	+
25.....	7.2	6.4	7.3	7.3	+	7.6	+
26.....	7.2	6.2	6.8	6.8	+	7.8	+
27.....	7.2	5.8	5.8	5.8	+	7.6	+
28.....	7.2	5.9	6.8	6.9	+	7.4	+
29.....	7.2	5.9	6.4	6.7	+	7.3	+
30.....	7.2	5.9	5.8	6.8	+	7.4	+
39.....	7.2	5.9	6.8	6.9	+	7.4	+
40.....	7.2	5.9	6.8	6.8	+	7.6	+
44.....	7.2	6.0	6.6	6.8	+	7.8	+
45.....	7.2	6.1	6.7	6.9	+	7.8	+

Table XIII gives the results in dextrin broth. These determinations were unsatisfactory because of the insolubility of the dextrin. Although several samples were tried, none were entirely satisfactory. *B. typhosus* seemed to attack this carbohydrate with difficulty. A slight transient acidity was produced in all cases with an alkaline reversion, beginning after forty-eight hours and continuing during the period of incubation. After one week the hydrogen-ion con-

TABLE XIV.—Summary of final hydrogen-ion concentration in meat-infusion broth containing 2 per cent of the fermentable carbohydrates.

ORGANISM.	2 per cent dextrose.	Growth, 72 hours.	2 per cent mannite.	72 hours.	27. Maltose.	72 hours.	27. Xylose.	72 hours.	27. Dextrin.	72 hours.
Average, 31 organisms	pH 4.9–5.1	0	pH 5.0–5.2	0	pH 5.1–5.6	+0	pH 4.7–5.2, except 21, 40 and 12	0	pH 5.6–6.6	+
No. 5	pH 5.1	+	pH 5.0	+	pH 5.2	+	alkaline	+	pH 6.6	+

+ No acid in seventy-two hours.

centration averaged 0.1-0.6 pH higher than the original reaction. All the organisms were viable.

No constant differences could be observed in the final hydrogen-ion concentration reached by the different strains and there were no differences which could be correlated with antigenic differences in dextrose, mannite, maltose and dextrin broth. The results in xylose broth are discussed separately.

Table XIV gives a summary of the results obtained using 2 per cent carbohydrate broth. In dextrose, the final hydrogen-ion concentration was practically the same as in 1 per cent broth. In mannite, maltose and xylose the hydrogen-ion concentration was slightly greater in 2 per cent than in 1 per cent broth. It was hard to account for this, since it was usually found that 1 per cent of the carbohydrate was not completely used. My later studies show that few of the organisms were able to use 1 per cent dextrose. Fred and Peterson (1920) found that *B. typhosus* gave the maximum acid yield in 2 per cent xylose although only 25 per cent of the sugar was used. In 2 per cent dextrin the hydrogen-ion concentration did not differ from that in 1 per cent broth.

TABLE XV.—Hydrogen-ion concentration in 2 per cent xylose broth.

ORGANISM.	Original 2 pH.	24 hours.	48 hours.	72 hours.	7 days.	14 days.	21 days.
12.....	6.914	6.864	7.472	7.574	6.340	5.714	5.528
5.....	6.914	6.948	7.760	8.132	8.487	8.470	8.419
21.....	6.914	6.999	7.591	7.506	6.982	6.779	6.627
40.....	6.948	6.661	6.948	7.067	7.236	6.593	5.866

Table XV gives the result of the hydrogen-ion concentration determinations in 2 per cent xylose broth of Nos. 5, 12, 21 and 40 after repeated transfers in xylose broth. It was hoped that the organisms might be trained to ferment xylose more rapidly. This was not accomplished.

(d) Viability in Broth Containing a Fermentable Carbohydrate.

With very few exceptions growth was tested for at seventy-two hours and thereafter whenever a hydrogen-ion determination was made. Summarizing our results, we find that when pH 5.0 to pH 5.2 was reached the organisms were rarely viable after seventy-two hours. If pH 5.4 to pH 5.5 was reached, the organisms were usually viable after one week, but many were not viable by the end of the

second week. When a lower hydrogen-ion concentration than pH 5.5 was reached, the majority were viable at the end of the third week. The organisms in 1 per cent mannite and maltose broth lived longer than in dextrose broth. This agrees with the work of Ishii (1920). The greater viability in carbohydrates other than dextrose is probably due to the higher hydrogen-ion concentration reached in dextrose broth. In 1 per cent xylose and dextrin broth where the hydrogen-ion concentration was lower than in dextrose, mannite or maltose, the organisms lived much longer. In 2 per cent mannite, maltose and xylose broth, where a greater concentration of hydrogen-ions was reached, the organisms were only slightly more viable than in dextrose broth.

The viability of the organisms could not be correlated with any antigenic differences noted previously except in the case of No. 5, which lived longer than the other strains. This viability is associated with the lower degree of hydrogen-ion concentration produced in most carbohydrates, with the more vigorous alkalin production and greater growth range.

(e) *The Influence of Varying Factors on the Final Hydrogen-ion Concentration in Dextrose Broth.*

Influence of varying amounts of glucose and the addition of buffers to the medium. Flasks of meat-infusion broth to which was added the percentage of dextrose desired were inoculated with .1 cc. of an eighteen-hour, second-generation broth culture of the different strains of *B. typhosus*. The reaction in twenty-four hours was tested by the colorimetric method. Four-tenths to six-tenths percent of dextrose was required to give the characteristic limiting hydrogen-ion concentration in meat infusion broth and from 0.6-0.8 per cent in meat-infusion broth to which dipotassium hydrogen phosphate had been added to act as a buffer. The final hydrogen-ion concentration was seen to be the same, although it is obvious that more acid must have been produced in the buffered than in the unbuffered broth. No significant differences between the strains could be found.

DISCUSSION.

The final hydrogen-ion concentration may be defined as the degree of acid produced by an organism in a utilizable carbohydrate. Jones, F. S. (1920), Jones, H. M. (1920), Dernby and Näslund (1923), called attention to the necessity for defining the conditions under which this final hydrogen-ion concentration is obtained. The

initial acidity, the kind and amount of carbohydrate, the age and amount of inoculum and the buffer effect of the medium all affect the limiting hydrogen-ion concentration. Levine (1917) points out that the final hydrogen-ion concentration represents not only the amount of acid formed from the carbohydrate fermented, but that it represents the sum of the metabolic activities of the organisms under the conditions stated.

The questions involved in this discussion are as follows: First, the effect of the carbohydrate on the final hydrogen-ion concentration attained; second, the constancy of the final hydrogen-ion concentration in any one carbohydrate; third, the amount of dextrose needed to produce the final hydrogen-ion concentration and the influence of buffers; fourth, the acid death point and its relation to the final hydrogen-ion concentration; fifth, the alkaline reversion observed after the final hydrogen-ion concentration is reached; sixth, the variations in the above reactions between the individual strains and the relation of such variations to antigenic differences.

FINAL HYDROGEN-ION CONCENTRATION IN DIFFERENT SUGARS.

The sugars used by the author were selected because they represent those most commonly used for the identification of *B. typhosus* and should be representative of the fundamental functional activity of this organism. Dextrose and maltose are always fermented by *B. typhosus;* mannitol is usually fermented. Hulton-Fraenkel (1919 *b*) reports that no acid formation took place in her synthetic medium to which mannite was added. I would suggest that this might have been due to slight growth. Ecker (1922) reports the occurrence of a serologically atypical strain of *B. typhosus* which did not produce acid in mannite. Xylose is usually fermented by *B. typhosus.*

There are many interesting observations in the literature as to the amount of acid produced by any one organism in different fermentable carbohydrates. It would seem that if acidity is the functional ability of the organism to resist acid, the same degree of acidity should be reached in any fermentable carbohydrate. That this may not be true may be seen from the following review and from my own results.

Broadhurst (1913), Smillie (1917), Jones (1918 *a* and *b*), Thro (1915) and Ayres (1918) record a variation in the final hydrogen-ion concentration reached by streptococci in different fermentable carbohydrates. Oppenheim (1920) records the greatest variation in

acid fermentation from streptococci in mannite broth. Julianelle (1922), using hemolytic staphylococci, observed variation in acid production in various carbohydrates. Bengston (1919) studying *B. vulgaris,* records the greatest acidity from the fermentation of dextrose. Fisher (1918) and Michaelis and Marcora (1912) observed that *B. coli* produced greater acidity in dextrose than in lactose broth. Browne (1914) using *B. coli,* correlates the amount of acid produced with the complexity of the sugar fermented. He suggests that the lower acidity in complex sugars is the result of some other decomposition product which accompanies the acids, and in connection with them is able to limit growth. Hall and Randall (1922) report the same acid limits in any fermentable carbohydrate using *B. welchii.* Avery and Cullen (1919 *a* and *b*), Ayers and Mudge (1922), have maintained that the final reaction is the same whenever the carbohydrate is utilizable at all and the conditions of fermentation are comparable. Comparative studies of the fermentation of many strains of organisms in different carbohydrates have not been made except in the case of the streptococci and *B. coli.* As a rule the differences in fermentation between different strains of the same organisms have not been significant.

The final hydrogen-ion concentration reached by *B. typhosus* in dextrose broth is given in the literature as follows: Kendall (1923), pH 5.0; Yuri (1923), pH 4.7 to 5.0; Hulton-Fraenkel (1919*b*), pH 6.5; Kligler (1916), pH 5.0 to 4.8 in peptone water, buffered, pH 4.6 to 4.8 in peptone water, without buffer; Winslow, Kligler and Rothburg (1919), pH 4.9. The last mentioned authors give the final hydrogen-ion concentration in mannite broth as pH 5.1. The final hydrogen-ion concentration in other carbohydrate broths is not recorded.

My results agreed with most of the investigators quoted above in that a distinct difference in the final hydrogen-ion concentration was evident in the different carbohydrates. Dextrose and mannite appeared to be most available judging from the amount of acid produced. Maltose, xylose and dextrin were less available. Practically all investigators agree that the concentration of hydrogen-ions is higher in dextrose than in other carbohydrates. My results were entirely in agreement with this. The use of two per cent carbohydrate tended to lessen the difference in the amount of acid produced in dextrose, mannite and maltose, but not in xylose and dextrin. The utilization of xylose by the so-called slow-fermenters merits special comment. From thirty-two cultures three of my

strains fermented xylose slowly, and one, No. 5, was entirely unable to ferment xylose. This is probably an unusually large percentage of slow fermenters.

Teague (1920) and Morishima (1921) record that out of one hundred and fifteen strains of *B. typhosus* three only remained negative in xylose broth on the twenty-seventh day. When transferred repeatedly in xylose broth two strains became positive, one only remaining negative. All strains, on plating gave daughter colonies and by picking colonies showing acid on the xylose plates rapid fermenters might be selected. My results so far have failed to confirm those of the above authors. No. 5 has continued completely negative in xylose broth for thirty days after inoculation. It reached the same degree of alkalinity as if it were in plain broth. Nos. 12, 21 and 40 gave slight acid after one to twenty-one days. None of these cultures have been trained to ferment xylose rapidly by repeated transfers. They continued to be viable as long as if they were in plain broth.

My results suggest that the lower acidity in carbohydrate broth other than dextrose was not due to inhibition of growth, but rather that there was inability to make further use of the carbohydrate. The possibility that an equilibrium was reached in which basic products balance acid formation must be considered also. Levine (1916) points out that the final hydrogen-ion concentration is first a measure of resistance to acid in some cases as with *B. coli* in dextrose broth, or second, the point of equilibrium between the decomposing carbohydrate and its end products, as in *B. aerogenes* cultures in dextrose broth.

With the exception of No. 5 no constant nor significant difference in the final hydrogen-ion concentration were observed between the different strains in dextrose, mannite, maltose and dextrin. No correlation between antigenic differences and the hydrogen-ion concentration reached by the different strains could be found.

The organisms which fermented xylose easily likewise showed no significant variations. The slow fermenters, Nos. 21, 40 and 12 belonged to the same antigenic group and were antigenically related to the majority of the strains, although they differed from them in the rate at which xylose was fermented.

I am in agreement with Teague (1920), who feels that whenever an organism decomposes a carbohydrate it should be called a positive fermentation test regardless of whether the acid appears in twenty-four hours or after three weeks. Any fermentation test

should extend over a sufficient length of time so the strains which ferment the carbohydrate slowly would be found. In any negative fermentation test there is the possibility that simultaneous alkaline production may mask acid production. Quantitative sugar determinations obviate this difficulty.

CONSTANCY OF HYDROGEN-ION CONCENTRATION IN ANY ONE CARBOHYDRATE.

It is conceivable that all organisms in one group might be capable of producing a constant and characteristic hydrogen-ion concentration in any one carbohydrate under stated conditions. Jones, H. M. (1920), remarks on the constancy of the limiting hydrogen-ion concentration for streptococci, and also for *B. typhosus,* under stated conditions. Avery and Cullen (1919), using pneumococci, observed similar constancy in the limiting hydrogen-ion concentration for this group. The limiting hydrogen-ion concentration in dextrose broth has been considered sufficiently constant under certain conditions to form a method of distinguishing between streptococci of bovine and human origin. Avery and Cullen (1919), Jones (1920), Brown (1920), Ayers and Rupp (1922), and Fuller and Armstrong (1913), have observed constant differences between bovine and human strains of streptococci. Ayers, Johnson and Davis (1918) have proposed using the final hydrogen-ion concentration as a basis for distinguishing between pathogenic and nonpathogenic strains of streptococci. Clark and Lubs (1915) found the hydrogen-ion concentration of *B. coli* in dextrose broth sufficiently constant to make it the basis of their methyl-red test. Cole and Onslow (1916) suggest the use of phenol red to distinguish between *B. paratyphosus A* and *B* and *B. typhosus.* They found that when two-tenths per cent glucose in a casein digest broth is used, in twenty-four hours *B. typhosus* and *B. paratyphosus A* gave a yellow or acid reaction, while *B. paratyphosus B* gave an alkaline or red color. From my results on buffers, it will be seen that such a reaction would hold true only for a medium in which the buffer content was slight. In a well-buffered medium *B. typhosus* might produce an alkaline reaction also in twenty-four hours if only two-tenths per cent of dextrose were present. Koser (1921) shows that in forty-eight hours animal strains of *B. paratyphosus* give pH 5.0 to 5.2, human strains give pH 5.6 to 5.8.

In a large series of experiments I found that the limiting hydrogen-ion concentration as reached by *B. typhosus* was remarkably

constant for each carbohydrate used provided that the amount of inoculum, the composition of the medium and the initial reaction in the different experiments were alike. There were no marked differences in hydrogen-ion concentration between the different strains in any one carbohydrate, with the exception of No. 5, which showed alkaline reversion in seventy-two hours. It was found possible to repeat these experiments many times, obtaining the same range of hydrogen-ion concentration for the different strains each time, providing the other factors were constant.

Yuri (1923) reports a pH of 4.7 to 5.0 for *B. typhosus* in dextrose meat-infusion broth. I found that only occasionally did any of my strains give a hydrogen-ion concentration as high as pH 4.7. Kendall (1921) reports that his strains of *B. typhosus* reached pH 5.0. This agrees with my results. A comparison of *B. typhosus* with other organisms shows that bovine streptococci have a limiting hydrogen-ion concentration in dextrose of pH 4.5 to 4.9; human streptococci pH 5.1 to 5.5; pneumococci pH 5.0; that is to say, these cocci give a higher concentration of hydrogen-ions in the case of bovine streptococci than *B. typhosus* and the human strains about the same as *B. typhosus*. Sherwood* found that a large series of *B. enteritidis* organisms from influenza cases gave a final hydrogen-ion concentration of pH 4.7 to 5.0. Others have reported that the paratyphoid group had a final hydrogen-ion concentration of pH 4.6 to 4.9; that is, slightly more acid than *B. typhosus*. Yuri (1923) reports several strains of *B. dysenteriæ* as producing a final hydrogen-ion concentration of pH 5.2 to 5.6 in dextrose meat infusion broth. This is slightly less acid than most of my strains of *B. typhosus*. The figures for *B. coli* are given from pH 4.5 to 4.8 in dextrose and from pH 4.8 to 5.0 in lactose. The final hydrogen-ion concentration in lactose is more nearly comparable then, to that of *B. typhosus* than it is in dextrose. It may be seen from the foregoing that the final hydrogen-ion concentration of organisms of different or related species is very similar in dextrose broth. Figures for the final hydrogen-ion concentration produced by various species in other carbohydrates which are fermentable by *B. typhosus* are not available for comparison.

I found that the final hydrogen-ion concentration of my different strains was quite constant in each of the different carbohydrates used with the possible exception of dextrin and xylose. In dextrose the final hydrogen-ion concentration was from pH 4.9 to 5.1; in

* Unpublished data secured by Dr. N. P. Sherwood in this laboratory.

mannite, pH 5.1; in maltose, pH 5.3 to 5.5. This final hydrogen-ion concentration for the various carbohydrates differed to the extent of 0.1 to 0.2 pH in different tests. None of the strains, with the possible exception of No. 5, had a tendency to give a consistently higher or lower hydrogen-ion concentration in any series of determinations. Therefore, there were no differences here which could be correlated with antigenic differences. No. 5, however, consistently produced less acid than the other strains. This strain also differed antigenically. In dextrin the sample of carbohydrate used affected the final hydrogen-ion concentration to a marked degree. An organism giving good acid production in one test might give little acid in a subsequent test. It was impossible to correlate these variations with other characteristics, and it was felt that extraneous factors which could not be controlled were largely responsible for these differences. In the case of xylose fermentation those organisms which attacked xylose readily reached a final hydrogen-ion concentration of pH 5.2 to 5.6 without showing any significant variations between the strains nor any correlation with observed antigenic differences.

One may conclude, then, that the final hydrogen-ion concentration in any one carbohydrate is constant for that carbohydrate, provided that other factors such as the composition of the medium, size of the inoculum, initial acidity and so forth are adequately controlled. This statement applies more particularly to the easily available carbohydrates such as, in the case of *B. typhosus,* dextrose, mannite, maltose, and in the majority of cases xylose, also.

THE AMOUNT OF DEXTROSE NECESSARY TO PRODUCE THE FINAL HYDROGEN-ION CONCENTRATION AND THE INFLUENCE OF BUFFERS AND INITIAL ACIDITY ON THE FINAL HYDROGEN-ION CONCENTRATION.

The amount of sugar necessary to produce the final hydrogen-ion concentration has been investigated by numerous workers. Avery and Cullen (1918), working with human and bovine streptococci, found that the final hydrogen-ion concentration was reached in broth containing 0.5 to 1.5 per cent dextrose. H. Jones (1920) found 0.2 per cent dextrose sufficient to give the characteristic limiting hydrogen-ion concentration for streptococci. Foster (1921*b*) found that 0.3 per cent dextrose was necessary for the production of the final hydrogen-ion concentration in cultures of *Streptococcus hemolyticus.* Browne (1914), using *B. coli,* found that with less than 1.0 per cent dextrose, varying amounts of titratable acid were obtained, but that the amount of acid was quite constant when the

percentage of dextrose was more than 1 per cent. Clark and Lubs (1915) found that *B. coli* required 0.5 per cent dextrose in order to reach its characteristic hydrogen-ion concentration and that if sufficient dextrose was present to give this hydrogen-ion concentration there was no marked alkaline reversion. Fred, Peterson and Davenport (1919) found that *B. typhosus* and other xylose fermenting organisms gave the largest acid yield in 2.0 per cent xylose broth. H. Jones (1920) found that 0.2 per cent dextrose would yield the characteristic final hydrogen-ion concentration with *B. typhosus*, *B. paratyphosus A*, and *B. vulgaris*. My results do not entirely agree with his. It is possible that his broth required less glucose to reach the final hydrogen-ion concentration because of its lower buffer content. He states that "ordinary broth is used." That this buffer factor is a very important one in bacteriological culture media has long been recognized. Henderson and Webster (1907) suggest the use of phosphates to preserve the neutrality of media during the growth of acid or alkali-forming organisms. Clark (1922*a*) has pointed out the great importance of buffers in culture media. Clark (1915*b*) showed that *B. coli* produces a somewhat higher concentration of hydrogen-ions in more highly buffered media. Kligler (1916), Berman and Rettger (1918), Bronfenbrenner and Schlesinger (1918*b*) have noted that the amount of acid produced depends on the concentration of carbohydrates, buffer and quantity of peptone used. Foster (1921*b*) notes that with dipotassium hydrogen phosphate used as a buffer, *Streptococcus hemolyticus* produced large amounts of acid, but did not reach its final hydrogen-ion concentration with less than 1.0 per cent of dextrose present.

Summarizing my experiment, then, I found that my organisms required from 0.4 to 0.6 per cent dextrose in meat infusion broth to give a characteristic final hydrogen-ion concentration of pH 4.9 to 5.1 in twenty-four hours. This was slightly higher than the figure given by others for various organisms working under different conditions. It is suggested that the natural buffers of meat infusion broth may account for this. In buffered broth from 0.8 to 1.0 per cent dextrose was needed to give the final hydrogen-ion concentration under the conditions of the test. It is impossible to make a close comparison between these results and those obtained with other organisms, as there appears to be little published on this subject.

My results showed that almost twice as much dextrose was needed to give the characteristic hydrogen-ion concentration in buffered as

in unbuffered broth. An adequate explanation of this is that it is due to a suppression of free-hydrogen-ions by the action of the buffer. The organisms used four-tenths to six-tenths per cent dextrose in unbuffered broth before producing the final hydrogen-ion concentration. This is a larger figure than is given by most authors. One explanation of this is that meat infusion broth is a highly buffered medium. Much of the work quoted was done with peptone water. This I have found to be poor in buffers and not a particularly suitable medium for the growth of *B. typhosus*.

H. Jones (1920) notes that the initial acidity has little to do with the final hydrogen-ion concentration, provided that it is in the optimum range for growth. Schoenholz (1921) warns us of the disproportionate changes taking place near the acid or alkaline limits of the hydrogen-ion concentration admitting growth. In my experiments the initial acidities varied from pH 7.0-7.5 but were well within the optimum zone of growth as shown in experiment I.

The initial reaction of the medium had little or no effect on the final hydrogen-ion concentration provided it was within the range of optimum growth.

ACID DEATH POINT.

Work on the acid death point of bacteria has been attacked from two angles—the disinfectant action of acids added to cultures, and the acidity produced by an organism as a result of its own fermentation activities. Winslow and Lockridge (1906) give a comprehensive review of the action of acids and salts as disinfectants. They found *B. typhosus* to be very susceptible to dilute acids and the toxicity of the acid depended on the free H-ions in the case of inorganic acids. Using organic acids, acetic and benzoic, the disinfectant action of the H-ions was complicated by a toxic action due to the anion and undissociated molecules. The presence of peptone decreased the disinfectant action of the acids and the authors suggest it may be due to a loose protein-acid combination. Since this work, a great deal has been done showing that the presence of buffers, such as various proteins and salts, decrease the toxicity of the hydrogen-ions. Bruenn (1915) found that the disinfectant action of lactic and acetic acids for *B. coli* and *B. typhosus* was due to the activity of the hydrogen-ions. Clark (1916) emphasizes the fact that it is the hydrogen-ion concentration and not the total amount of acid that limits growth. Michaelis and Marcora (1912) discuss the limit of acid production by *B. coli* and the maximal acid production which is compatible with life. They also point out that the

injury is due to the hydrogen-ion. They found that acetic acid was toxic in a concentration of from 4.10^{-5} to 8.10^{-5}. McDonald (1917) found that *B. typhosus* could withstand three-tenths per cent of lactic acid. His results are greatly complicated by the addition of glucose to his test medium, and since *B. typhosus* ferments glucose with the production of both lactic and acetic acids, it is hard to evaluate his results. Rambousek (1900) found *B. typhosus* is less resistant to lactic acid than is *B. coli.* Kitasato (1888) recognized that the action of different acids depends on the medium and the age of the culture used for inoculation. Duchacek (1904) found that *B. typhosus* produces about equal quantities of acetic and lactic acids, possibly more lactic acid than acetic. Harden (1901) found that *B. typhosus* produces lactic, acetic and succinic acid, ethyl alcohol and a considerable amount of formic acid. Sera (1910) found that *B. typhosus* produces acetic acid and a trace of alcohol. Thus it may be seen that the acids produced by the metabolism of the organisms may have disinfectant action.

My experiments on the viability and the final hydrogen-ion concentration show that where pH 4.9-5.2 is reached the organisms are generally dead in seventy-two hours. This corresponds to the figures given by Ishii (1920) for glucose broth. More of my organisms remained viable after seventy-two hours in mannite than they did in glucose. Sera (1910) has pointed out that *B. typhosus* produces less acetic acid from mannite than from glucose. This may be a factor in the greater viability in mannite.

In maltose, dextrine and xylose the organisms did not produce so much acid and remained viable over greater periods of time. Two factors may be concerned, namely, the greater viability may be due to the smaller concentration of hydrogen-ions or to the production of less toxic by-products. Fred and Peterson (1920) found that *B. typhosus* produced formic, acetic, butyric and succinic acids, ethyl alcohol and a very small amount of CO_2 from xylose. Only twenty-five per cent of the xylose was used. They note that the reason for the use of such a small amount of xylose is not known. Burton and Rettger (1917) suggest that the cessation of growth of organisms is due to the end products or an equilibrium of the enzymes produced. Clark (1915 *b*), Winslow, Kligler and Rothberg (1919), and Browne (1914) point out that some of the limiting factors may be due to toxic products other than acids produced during growth.

My results were examined with a view to ascertaining whether

there were any strains which consistently showed a resistance to the acids produced. With the exception of No. 5, if the strains were able to produce a hydrogen-ion concentration of from pH 4.9 to 5.2 in a fermentable carbohydrate they were not viable after seventy-two to ninety-six hours. No. 5 did not, as a rule, produce as much acid as the other strains, but it was also more acid resistant. This is borne out by the experiment showing the growth range of the different organisms.

It may be seen from the foregoing that in dealing with the limiting hydrogen-ion concentration of any organism, the following factors must be considered: First, the concentration of hydrogen-ion; second, the acids produced by the fermentative activity of the organisms; third, other toxic substances produced during growth.

ALKALINE REVERSION.

A slight but definite change in hydrogen-ion concentration toward the alkaline side was noted in all cultures after seventy-two hours or longer. In those cases where the organisms remained viable, the alkaline reversion was more marked. Kendall (1921) notes this alkaline reversion after death of the organism and states that it is probably due to autolytic changes. It is accompanied by a decrease in protein-nitrogen and an increase in non-protein nitrogen. Hall and Randall (1922) state that a fall in acidity of the medium is not proof that the medium is free of sugar. They suggest that acid may be changed to less dissociable compounds. Fischer (1913) noted this alkaline reversion and suggested that it might be due to binding of the acid or to the production of bases. Ayers and Rupp (1918 *a* and *b*) in their explanation of the methyl-red reaction with *B. coli* and *B. aerogenes,* indicate clearly that a simultaneous decomposition of the acid by products independent of ammonia production may take place with a consequent alkaline reaction.

Levine (1916 *a* and *b*) states that if, after the limiting hydrogen-ion concentration is reached by an organism, it remains viable, it will attack the proteins, forming alkali which neutralizes some of the acid and enables it to use more carbohydrate.

The alkaline reversion considered above should be distinguished from the progressively alkaline reaction produced in a culture medium which contains no available carbohydrate. The tendency to slight alkaline reversion was noted in all the strains and was not correlated with any antigenic differences observed. No. 5, how-

ever, always produced a more marked alkaline reaction than the other organisms.

There was, however, a decided tendency toward a greater alkaline reversion in the order of availability of the carbohydrate used. Dextrose gave the greatest concentration of hydrogen-ions and showed the least reversion, never more than 0.4 pH. Mannite was practically the same as dextrose. Maltose gave a lower concentration of hydrogen-ions and showed a reversion of from 0.4 to 1.0 pH. Dextrin gave the lowest hydrogen-ion concentration values and the reversion varied from 0.6 to 1.9 pH. The rapid xylose-fermenters gave an average pH of 5.2 to 5.6 and an alkaline reversion from 0.3 to 1.0 pH.

No. 5, which produced no acid from xylose, gave a progressively alkaline reaction. The slow xylose-fermenters gave an alkaline reaction, then a slight acid reaction followed by an alkaline reversion. It would seem in this case that the organism was able to use only a very small amount of the xylose and that the alkaline reaction was due to the subsequent utilization of proteins.

SUMMARY.

The findings of the foregoing experiments may be summarized as follows:

1. The range of growth in plain meat infusion broth of the strains of *B. typhosus* used was from pH 5.0 to pH 9.0.

2. There was a slight increase in titratable acidity in broth to which no carbohydrate had been added. The reaction after twenty-four hours became increasingly alkaline.

3. The final hydrogen-ion concentration reached in dextrose, mannite and maltose broth was characteristic for the carbohydrates used and was constant under stated conditions. This was also true for xylose in ninety per cent of the strains. The final hydrogen-ion concentration in dextrin was variable and appeared to depend somewhat upon the solubility of the sample of dextrin used.

4. A definite amount of dextrose was necessary to give the characteristic limiting hydrogen-ion concentration. This amount was larger if a broth rich in buffers was used.

5. The initial hydrogen-ion concentration of the carbohydrate used did not affect the final hydrogen-ion concentration provided the initial hydrogen-ion concentration was in the optimum range of the organisms.

6. In broth containing a fermentable carbohydrate, if pH 4.9-

5.2 was reached, the organisms were rarely viable after seventy-two hours.

7. The kind of carbohydrate fermented determined to a certain extent the viability of the organisms after the final hydrogen-ion concentration was reached.

8. There was a distinct alkaline reversion present in all cultures twenty-four to forty-eight hours after the final hydrogen-ion concentration was reached. This reversion was least apparent in dextrose and increased in the different carbohydrates in the order named —mannite, maltose, xylose and dextrin.

9. No noteworthy differences between the different strains was apparent, nor could any relation be discovered between the reactions studied here and the antigenic differences exhibited by these organisms. No. 5 is an exception to the above statement.

QUANTITATIVE TESTS.—EXPERIMENTS ON THE UTILIZATION OF DEXTROSE AND ITS RELATION TO THE BUFFER CONTENT OF THE MEDIUM; TO THE TITRATABLE ACID; TO THE GROWTH RATE AND THE FINAL HYDROGEN-ION CONCENTRATION.

The importance of the interrelation between the sugar utilized, the buffer effect of the medium and the acids produced has engaged the attention of bacteriologists for many years. Kitasato (1888) recognized that the effect of acids on an organism was greatly influenced by the composition of the medium. Iwanow (1892) using various organisms, studied the formation of acids in milk and peptone-lactose water, finding that there was twice or three times more acid produced in milk than in peptone water.

Recently there has been a great deal of work done on streptococci to determine the final hydrogen-ion concentration. Since this is so intimately bound up with the amount of dextrose used and the buffer effect of the medium these factors are also considered in these articles.

Fuller and Armstrong (1913) found that over 3.5 per cent acid is produced so uniformly by human strains of fecal streptococci that this may serve to distinguish them from fecal streptococci from animal sources.

Jones, F. S. (1918 *a* and *b*), studied the amount of titratable acid in carbohydrate broth and its relation to the source of the streptococci used. The same author (1920) showed that the kind of media used and the buffer content of the media exerted an effect on the limiting hydrogen-ion concentration. He found that peptone greatly

increased the amount of acid produced, probably due to its buffer action.

Ayers and Rupp (1922) found that the hydrogen-ion concentration of the media and the amount of buffer present exerted an effect on the hippurate-splitting enzyme of hemolytic streptococci.

Avery and Cullen (1919 *a* and *b*) showed that 0.5-1.5 per cent dextrose was sufficient to give the final hydrogen-ion concentration of *Streptococcus hemolyticus* in meat infusion broth. No change in reaction occurred after the first twenty-four hours. These same authors (1919) working with pneumococci found that 0.4 per cent dextrose was necessary to give the final hydrogen-ion concentration. The organisms did not reach their final hydrogen-ion concentration in nine hours, but did in twenty-four hours. Lord (1919) showed that there was rapidly increasing acidity in pneumococci cultures accompanied by rapid growth.

Julianelle (1922) found that staphylococci do not use 0.5 per cent dextrose in peptone water containing 0.5 per cent dipotassium hydrogen phosphate. Grace and Highberger (1920) using *Streptococcus viridans* found that the final hydrogen-ion concentration does not vary in a medium containing from 0.2 per cent to 1.0 per cent dextrose.

Ayers, Johnson and Davis (1918) emphasize the fact that titratable acid is influenced so much by the nature and buffer content of the medium that this method should be supplanted by hydrogen-ion determination.

Pere (1892), using *B. typhosus* and *B. coli,* recognized that a lag in acid formation occurred unless the inoculum was young. He found that the amount of acid depended on the amount of sugar present. *B. typhosus* removed ninety per cent of dextrose in a digested egg medium in fourteen days, and *B. coli* did not entirely use 5.0 per cent lactose in eight days.

Tissier (1902) recognized that *B. coli* produced acid from dextrose and that protein was not so readily attacked in dextrose medium. He also states that the alkaline reversion which took place in some cases was due to an insufficient amount of sugar. He estimated that 0.173 per cent normal sulphuric acid was necessary to limit the growth of *B. coli.* He also suggests that certain strains of staphylococci may be able to attack proteins and dextrose simultaneously.

Kendall (1913) points out that the progressive acidity in a carbohydrate-containing medium is due to the utilization of the

carbohydrate for energy by the rapidly growing organisms. If the carbohydrate may be entirely used without producing enough acid to limit the growth of the organism, they then attack the proteins with the production of ammonia and an alkaline reversion of the medium. On the other hand, in a carbohydrate-free medium the only source of energy resides in the protein molecule. The ammonia is split off prior to the utilization of the nitrogen-free remainder of the molecule for energy and the reaction of the medium becomes progressively alkaline. Kendall then points out that fermentation takes precedence over putrefaction.

Observations on the reaction of the medium, therefore, give a certain amount of information concerning the ability of the organisms to utilize carbohydrates. This ability again hinges on the buffer effect of the medium. It has been assumed by some authors that a progressively alkaline reaction presupposed an inability on the part of the organism to utilize the carbohydrate present. That this is not the case is shown by DeBord (1923) in his studies on *B. pyocyaneus*. Quantitative dextrose determination showed a steady diminution in dextrose in spite of the increasing alkalinity of the medium. This alkalinity may be due to the further utilization of the acids produced from the dextrose or to the neutralization of the acids by the basic products of proteolysis. Sherwood (1926), using *B. pyocyaneus*, showed that certain of his strains agreed with the findings of DeBord (1923), but that in a number of strains, glucose utilization was accompanied by increasing acidity. The work of Ayers and Rupp (1918) has also shown that dextrose may continue to be utilized in spite of the production of an alkaline reaction by *B. aerogenes*.

Levine (1916) points out that if the organisms remain viable after the final hydrogen-ion concentration is reached, they attack the protein constituents, forming basic substances which neutralize the acids and allow the formation of more acid from the remaining carbohydrate.

Glenn (1911) found that the amount of acid produced in any medium was the function of the medium rather than the function of the organism.

In the following experiments an attempt was made to correlate the following factors:

1. The rate and total amount of dextrose used by *B. typhosus* in buffered and unbuffered broth.
2. The rate and amount of acid produced.

3. The rate and extent of the change in hydrogen-ion concentration.

4. The rate of growth of *B. typhosus*.

5. Comparison of the metabolism of the strains used to one another and to the serological differences exhibited by these strains as demonstrated in Part I.

METHODS.

In the following experiments determinations of glucose, titratable acidity, number of organisms and hydrogen-ion content were made on a buffered and unbuffered medium at three-hour intervals for twelve hours and thereafter at 24-, 48- and 72-hour periods. Many determinations were done at seven-day and fourteen-day periods, but these are not recorded in the tables, as no significant change occurred after the death of the organisms, which usually took place before the fourth day. If the determinations were continued after the fourth day, it was necessary to weigh the flasks, and after incubation make up the loss due to evaporation by adding distilled water.

MEDIA. The unbuffered medium was meat-infusion broth, to which the dextrose was added in sterile solution.

The buffered medium was meat-infusion broth, to which was added 16 cc. of dihydrogen potassium phosphate and 144 cc. disodium hydrogen phosphate for every 1,500 cc. of medium. The dextrose was added as above.

The broth was adjusted to a slightly alkaline reaction by the colorimetric method. In many cases, the hydrogen-ion concentration was checked by the electrometric method.

The inoculum consisted of 0.2 cc. of a third-generation eighteen-hour broth culture. The number of organisms was determined by plating, in duplicate, 1 cc. of the medium in proper dilutions in plain agar adjusted to pH 7.0-7.5. The plates were incubated for seventy-two hours and the colonies counted. The figures given in each case are the logarithm of the number of organisms found.

The Folin titration method was used at first for the dextrose determinations. This method proved to be troublesome and subject to several sources of error. The broth bumped and spattered badly even when all reasonable precautions were used to prevent this. As the solution can be boiled only six minutes without danger of destroying the sharpness of the end point, it was difficult to do this when the broth spattered so badly. When two or three successive

titrations could be checked within reasonable limits, the results could be accepted, but the technical troubles mentioned made such checks difficult and time consuming. The final method used was the Folin-Wu colorimetric method for blood-sugar determination.

A protein-free filtrate was prepared using the method given for preparation of a filtrate from blood, Cole (1919). Aliquot parts of the clear filtrate were diluted in volumetric flasks, a 1-50 or 1-100 dilution being satisfactory in most cases. Two cubic centimeters of this dilution were placed in Folin tubes with the alkaline-copper solution and treated as for blood filtrates. Two-tenths milligram and four-tenths milligram standard glucose solutions were prepared in the same way. The comparison was made with the most suitable standard in a Dubosque colorimeter. All tests were made in duplicate. A comparison of the titrametric and colorimetric methods showed that the titrametric method was as accurate as the colorimetric method, but in the long run it was more time consuming and because of the difficulties mentioned above it was less satisfactory than the colorimetric method.

The titratable acidity was determined by titration of duplicate or triplicate 5 cc. quantities of the broth diluted with 45 cc. of distilled water. Titration was done with N/20 NaOH, using phenolphthalein as in indicator. The titratable acidity is expressed in number of cubic centimeters of acid per 100 cc. of medium. Berman and Rettger (1918*a*), Jones, F. S. (1920), and others have demonstrated that it is not necessary to subtract the initial titratable acidity from the final titration.

The hydrogen-ion concentration of the broth was determined by the colorimetric method in many cases and in others by the electrometric method. The limits of error between the two methods was never more than plus or minus .1 pH.

RESULTS.

(*a*) *Utilization of Dextrose.*

The following tables give the amounts of dextrose used by those organisms showing the greatest amount of variation. As a rule the differences observed between the different strains were no greater than those observed in different determinations of the same strains.

TABLE XVI.—Utilization of glucose in unbuffered broth.

ORGANISMS.	Original sugar.	0 pH.	3 hours.			6 hours.			9 hours.		
			Sugar.	Sugar used.	pH.	Sugar.	Sugar used.	pH.	Sugar.	Sugar used.	pH.
2	1.219	7.0	1.179	.040	7.0	1.179	.051	6.8	1.004	.219	6.1
5	1.219	7.0	1.136	.083 (6%)	7.1	1.136	.083	6.8	1.037	.183	6.2
1	1.2690	7.0	1.240	.0290	7.0	1.1848	.0842	6.8	1.0822	.1868	6.4
21	1.3513	7.0	1.2690	.0823	7.0	.1904	.1609	6.9	.1106	.2452	6.0
3	1.0460	7.6	1.0282	.0278	7.5	.8417	.2043	5.5	.7418	.3042	5.3
12	1.2690	7.6	1.2315	.0370	7.4	1.1674	.1016	6.8	1.0632	.2058	5.3
13	1.1240	7.8	1.1164 1.1108	(6%) .0076 .0132	7.2	.9192 .9192	(10%) .2208 .2208	6.4	.8954	.2286 (20%)	5.3
4	1.0956	7.6	.9876	.1080 (9%)	7.4	.9756	.120 (10%)	5.6	.8608	.2348 (21%)	5.6
14											

TABLE XVI.—Utilization of glucose in unbuffered broth—*Concluded.*

ORGANISMS.	12 hours.			24 hours.			48 hours.			72 hours.			Growth.
	Sugar.	Sugar used.	pH.	Sugar.	Sugar used.	pH.	Sugar.	Sugar used.	pH.	Sugar.	Sugar used.	pH.	
2	.8150	.408	5.7	.6852	.5338	5.1	.6352	.5838	5.1	.6133	.6057	5.3	—
5	.9350	.2840	5.8	.8563	.3627	5.3	.8146	.4044	5.3	.7331	.4859	5.5	+
1	.9804	.2886	5.9	.8143	.4547	5.2	.6495	.6195	5.3	.5896	.6794	5.3	+
21	.8333	.5180	5.6	.7000	.6511	5.1	.6381	.7132	5.1	.5760	.7753	5.3	+
3	.6906	.3554	5.2	.6250	.4210	5.0	.6022	.4438	5.4	—	—	5.4	—
12	.9956	.2734	5.2	.7278	.2884	5.1	.5630	.4532	5.3	.5176	.4986	5.2	—
13	.8950	.2290 (20%)	5.1	.8552	.2680	5.1	.8550	.2682	5.1	.8314	.2918	5.2	—
4	.8080	.2876 (26%)	5.3	.7652	.3304	5.1	.7063	.3893	5.241	.7064	.3892	5.2	—
14	.....	.....	.....	.8230 .8126	.3344	5.0	.7662 .7421	.3999	5.0	.7119 .7168	.4372	5.1	0

TABLE XVII.—Utilization of glucose in buffered broth by three-hour intervals.

ORGANISM.	Original sugar.	0 pH.	3 hours.			6 hours.			9 hours.		
			Sugar.	Sugar used.	pH.	Sugar.	Sugar used.	pH.	Sugar.	Sugar used.	pH.
2	1.1013	7.3	1.0204	.0809	7.3	.6660	.4353	7.2	.5880	.5133	5.6
5	.9048	7.7	.....	.....	.....	.8602	.0446	7.0	.8032	.1016	6.5
1	1.0683	7.5	1.0204	.0434	7.5	.9920	.0718	7.3	.9259	.1379	6.8
21	1.1061	7.3	1.0775	.0286	7.3	.7016	.4045	7.3	.6140	.4921	6.2
3	1.0638	7.5	1.0593	.0045	7.5	.9259	.1379	7.3	.5924	.4714	6.5
12	.8000	7.5	.5680	.2320	7.5	.5400	.2600	7.2	.1106	.6894	6.3
13	.8000	7.5	.5480	.2520	7.5	.4680	.3320	7.1	.0980	.7020	6.2
4	1.080	7.4	1.0524	.0276	7.4	1.000	.0876	7.0	.6152	.4648	5.8

TABLE XVII.—Utilization of glucose in buffered broth by three-hour intervals—*Concluded.*

ORGANISM.	12 hours.			24 hours.			48 hours.			72 hours.			Growth.
	Sugar.	Sugar used.	pH.	Sugar.	Sugar used.	pH.	Sugar.	Sugar used.	pH.	Sugar.	Sugar used.	pH.	
2	.5320	.5693	5.8	.3538	.7475	5.0	.3508	.7505	5.1	.3418	.7595	5.1	0
5	.7016	.2032	6.5	.3498	.5550	5.3	.2596	.6452	5.3	.2450	.6598	5.5	+
1	.5800	.4838	5.7	.3918	.672	5.3	.3538	.710	5.3	.3538	.710	5.3	0
21	.4640	.6421	5.6	.3148	.7913	5.1	.3148	.7913	5.1	.3148	.7913	5.1	0
3	.....	.....	5.3	.....	.....	5.2	.4494	.6144	5.3	.3572	.7066	5.3	0
12	.0586	.7414	5.8	.04410 .0727	.7559 .6077	5.4	.0450 .0454	.7568 .6350	5.6	*.....	*.....	5.6	+
13	.0638	.7362	5.8	.0432	.7568 .7252	5.3	.4596	.7252	5.6	.4444	.7404	5.6	+ +
4	.4968	.5832	5.2	.3720	.7080	5.0	.3720	.7079	5.0	.3718	.7082	5.3	0

* Too small to be determined.

Table XVI gives the change in hydrogen-ion concentration and utilization of sugar by three-hour periods for the first twelve hours and thereafter at 24-, 48- and 72-hour periods, using meat-infusion dextrose broth without the addition of phosphates. It will be seen that in most cases the largest amount of sugar was used between the sixth and twelfth hours. Up to the third hour, very little sugar was used; the amount averaged 0.4 gram. From the third to the sixth hour, the consumption increased, giving an average of 0.09 gram used. From the sixth to the ninth hours 0.13 gram was used, and from the ninth to the twelfth 0.1 gram. There was a marked decrease in the consumption of sugar from the twelfth to the twenty-fourth hour, the average amount used for three-hour periods was only 0.01 gram; from the twenty-fourth hour to the seventy-second hour there was usually a small amount utilized, from 0.005 to 0.23 gram, an average of 0.07 gram. Frequently a small amount of sugar was used between the twenty-fourth and forty-eighth hour, an average of 0.05 gram. Very little or none was used between the forty-eighth and seventy-second hour, an average of 0.02 gram. Strain No. 5 used from 0.04 to 0.05 gram between the twenty-fourth and forty-eighth hour, and from 0.08 to 0.12 gram between the forty-eighth and seventy-second hour as against 0.07 gram for the other organisms.

As long as the organisms remain viable there was a small amount of sugar used. Presumably this sugar served as a source of energy. The organisms after twenty-four hours were decreasing in numbers and only the minimum amount of carbohydrate was required. Except for strain No. 5 there were no constant differences observed between the organisms which could be correlated with their antigenic differences.

Table XVII shows the utilization of dextrose in a heavily buffered meat-infusion broth. Here there was greater irregularity in the rate and amount of sugar utilization. The amount of sugar used in the first three hours was greater than in the unbuffered broth. The amount used between the third and sixth hours was also greater, average 0.13 gram. Maximum utilization usually took place between the sixth and ninth hours in the buffered as well as in the unbuffered broth, an average of 0.23 gram being used: that is, almost twice that used in unbuffered broth. From the ninth to twelfth hours the amount of dextrose used decreased to 0.11 gram. The decrease in sugar utilization was therefore greater in the buffered than in the unbuffered medium. From the twelfth to the twenty-

fourth hour, the average consumption of sugar for three-hour periods was 0.04 gram. This was four times the amount per hour used by the organisms in unbuffered broth. From the twenty-fourth to the forty-eighth hour, very little dextrose was used, average 0.02 gram; that is, about one-half that used in unbuffered broth. There was practically no dextrose used between the forty-eighth hour and the seventy-second hour. A comparison between the average amount of dextrose used in the buffered and unbuffered broth by three-hour periods showed that the total quantity used in buffered broth was considerably greater than in unbuffered broth. The amount used in the first three hours was much greater in the buffered broth, while during the next three hours the amount used in buffered broth was only slightly greater than in unbuffered broth. The maximum utilization of dextrose in both buffered and unbuffered broth occurred between the sixth and ninth hours but the total utilization in buffered broth was greater during this period, being two and a half times that in unbuffered broth. The inhibition in dextrose utilization from the ninth to twelfth hour was more marked in buffered than in unbuffered broth. There was less utilization of dextrose after the twenty-fourth hour in buffered broth. This was probably a continuance of the inhibition noted after the ninth hour.

The average for these results may be seen in Table XVIII. The total amount of dextrose used in buffered and unbuffered broth is an excellent example of the effect of buffers on the utilization of dextrose. The organisms used from 75 to 100 per cent of the dextrose present in buffered broth, but only from 25 to 50 per cent of the dextrose in unbuffered broth.

As in unbuffered broth, no constant differences were observed in the dextrose utilization by the different strains, nor was there any correlation between the utilization of dextrose in buffered broth and antigenic differences with the exception of strain No. 5.

(b) *Change in Titratable Acidity and Hydrogen-ion Concentration in Buffered and Unbuffered Broth.*

Tables XIX and XX give the change in titratable acidity and hydrogen-ion concentrations of the organisms at three-hour intervals and thereafter at 24,- 48- and 72-hour periods in buffered and unbuffered broth.

The greatest increase in titratable acidity took place during the period when the dextrose was being most rapidly used. There was a slight increase in acidity up to the third hour. From the third to

the sixth hour the average increase was 1.62 per cent; from the sixth to the ninth hour 2.35 per cent, and from the ninth to the twelfth hour 0.91 per cent. From the twelfth to the twenty-fourth hour there was an increase in acidity averaging 0.19 per cent for each three-hour period. The increase in titratable acidity in the unbuffered broth up to the twenty-fourth hour was parallel to the utilization of dextrose. The changes in titratable acidity from the twenty-fourth to the seventy-second hour were somewhat irregular. In about fifty per cent of the determinations a slight decrease in titratable acidity took place between the twenty-fourth and the seventy-second hour. This decrease was more marked when the observations extended over a week. It was probably due to autolytic changes, since with the exception of No. 5 all of the organisms in unbuffered broth were dead by the seventy-second hour. The final reaction of the medium was from 6 to 8 per cent acid to phenolphthalein after twenty-four hours.

The change in hydrogen-ion concentration in unbuffered broth followed the change in titratable acidity and sugar utilization closely from the third to the twelfth hour, when the final hydrogen-ion concentration was usually reached. There was little or no change in hydrogen-ion concentration up to the third hour although dextrose was being used during this period. After the twelfth hour the hydrogen-ion concentration remained constant, although dextrose was being used and titratable acid was increasing. After twenty-four hours a slight alkaline reversion took place and was usually accompanied by a decrease in titratable acidity. Using strain No. 5, the decrease in titratable acid and the decrease in hydrogen-ion concentration was more apparent than with the other organisms. This organism was usually viable after seventy-two hours and continued to use dextrose in spite of the alkaline reversion. Aside from this there were no peculiarities noted for any of the individual strains used.

TABLE XVIII.—Analysis of percentage of sugar used in buffered and unbuffered broth by hours.

ORGANISM.	3 hours.		6 hours.		9 hours.		12 hours.		24 hours.		48 hours.		72 hours.	
	B	P	B	P	B	P	B	P	B	P	B	P	B	P
2	7%	3	30	4	46	17	51	33	67	44	68	49	68+	49+
5	—	6	4.9	7	11	14	22	23	61	30	71	—	72	39
1	4	2	6	6	13	14	45	22	63	35	66	—	66+	53
21	2 7	6	36 10	11	44 32	18	58 71	38	71 81	49	— 83	—	— 84	58
3	4	2	13	19	44	29	—	33	—	40	57	42	66	54
12	29	2	32	15	86	31	92	36	94 89	36	94 93	36	— 93	37
13	31	0.6	41	10	87	20	92	20	61	23	61+	23	62	25
4	2	9	8	10	43	21	54	26	65	31	65	31	65	35
Average	10	3.8	20	9.6	45%	20	60%	28	72	36	73	36	75	42

B.—Buffered broth.
P.—Unbuffered broth.

TABLE XIX.—Rate of acidity in glucose unbuffered broth.

ORGANISM.	Original number of organism.	pH.	3 hours.			6 hours.			9 hours.		
			Acid.	pH.	Organism log.	Acid.	pH.	Log.	Acid.	pH.	Log.
2	3.9031	7.0	2.2	7.0	4.0000	2.3	6.8	9.3010	5.22	6.1	9.6385
5	4.3979	7.4	2.02	7.3	6.2900	4.1+	6.8	8.2553	5.80+	5.7	9.0000
1	4.8451	7.4	2.80	7.4	5.3222	3.15+	6.7	8.3010	5.45	5.3	8.4472
21	6.4014	7.5	2.10	7.5	6.6021	2.37	7.1	7.3002	4.72	.....	8.8415
3	6.4281	7.5	2.00	7.5	6.6021	4.62+	6.5	7.4771	6.82	5.8	9.5682
12	4.7709	7.6	.7+	7.4	4.771	4.5+	6.8	6.6532	5.7	5.3	6.9542
13	5.6990	7.8	2.00	7.2	6.8808	4.0+	6.4	7.3010	6.7	5.3	8.4771
4	.21 3.9031	7.4	2.20	7.3	4.0000	2.6	6.7	9.3010	5.22	5.6	9.6385

TABLE XIX—Rate of acidity in glucose unbuffered broth—*Concluded.*

ORGANISM.	12 hours.			24 hours.		48 hours.		72 hours.		Growth.
	Acid.	pH.	Log.	Acid.	pH.	Acid.	pH.	Acid.	pH.	
2	6.67	5.7	10.7924	7.37	5.1	6.25	5.4	6.15	5.4	0
5	5.95	5.3	10.6532	6.50	5.3	7.14	5.3	7.00	5.3	0
1	6.65	5.2	9.0212	7.05	5.2	6.25	5.2	6.82	5.3	0
21	7.2+	5.3	11.6990	8.07	5.1	6.52	5.3	7.45	5.5	+
3	6.87	5.2	12.5416	7.37	5.0	7.20	5.2	6.5	5.2	0
12	7.4	5.2	8.8451	8.50	5.0	—	—	8.0	5.0	0
13	7.5	5.1	10.3010	7.50	5.1	—	—	7.62	5.2	0
4	6.67	5.3	10.7924	7.27	5.1	6.25	5.1	6.15	5.3	0

TABLE XX.—Rate of acidity in glucose buffered broth.

ORGANISM.	Original number of organism.	Original pH.	3 hours.			6 hours.			9 hours.		
			*Acid.	pH.	Organism log.	Acid.	pH.	Log.	Acid.	pH.	Log.
2	5.4624	7.3	2.5	7.3	5.7782	4.35	7.2	6.3032	12.10	6.6	9.1206
5	4.000	7.9	3.05		5.4800	3.25 3.05	7.0	7.4472	7.90	6.5	8.7543
21	5.8096	7.5	.212	7.2	6.0969	4.12	7.2	6.3892	4.87	6.2	9.7493
1	4.0170	7.8	1.5	7.3	5.4800	3.85	6.8	6.3892	9.2	6.7	9.5465
3	5.4624	7.5	2.50	7.5	5.7782	4.35	7.3	6.3032	12.10	5.5	9.4206
12	5.6232	7.8	2.20	7.8	5.3424	2.42	7.5	6.3010	10+	6.3	9.3010
13	5.4624	7.8	2.50	7.8	5.7709	4.75	7.1	6.2553	14.10	6.2	9.0934
4	6.0000	7.4	2.8	7.4	6.3979	4.95	7.0	7.4065	17+	5.8	7.415

* Acidity in terms of 2 per cent normal broth.

TABLE XX.—Rate of acidity in glucose buffered broth—*Concluded.*

ORGANISM.	12 hours.			24 hours.		48 hours.		72 hours.		Growth.
	Acid.	pH.	Log.	Acid.	pH.	Acid.	pH.	Acid.	pH.	
2	18.20	5.8	9.9961	21.2	5.0	21.0	5.1	20.5	5.1	0
5	13.17	6.5	9.5079	20.95	5.3	21.12	5.3	23.60	5.5	+
21	23.05	5.2	10.6990	25.00	5.0	24.12	5.0	20.82	5.1	0
1	14.9	5.3	9.9547	19.50	5.3	20.3	5.3	21.45	5.3	0
3	18.20	5.3	9.9961	21.2	5.2	21.0	5.2	20.5	5.3	0
12	12.0	5.8	10.6990	16.28	5.4	20.55	5.6	20.60	5.6	+
13	16.2	5.8	10.6990	23.75	5.3	20.00	5.6	20.05	5.6	+
4	18.0	5.2	8.959	21.0+	5.0	19.3	5.1	19.80	5.5	0

The titratable acidity in buffered broth also followed the utilization of dextrose very closely. Up to the third hour there was slight increase in titratable acidity; from the third to the sixth hour there was an average increase of 1.5 per cent; from the sixth to the ninth hour, that is, during the period of maximum sugar utilization, there was an average increase of 6.7 per cent; from the ninth to the twelfth hour, an increase of 5.7 per cent, and from the twelfth to the twenty-fourth hour an increase of 4.5 per cent. From the twelfth to the twenty-fourth hour, the average increase for each three-hour period was 1.1 per cent. During this period, then, there was apparently a disproportionate amount of acid present. This is also noted by Jones, F. S. (1920). The dextrose is being used in diminishing amounts, but the acids continue to accumulate in the medium. From the twenty-fourth to the seventy-second hour there was little or no increase in titratable acidity. This seems to be due to the fact that very little dextrose was being used during this period.

Strain No. 5 continued to show an increase in titratable acidity up to the seventy-second hour in buffered broth with a gradual decrease from this time on. The utilization of dextrose by No. 5 with an increase in titratable acidity, continued for a longer period of time in buffered than in unbuffered broth.

With all strains the change in hydrogen-ion concentration was much slower in buffered than in unbuffered broth. The change in hydrogen-ion concentration up to the third hour was not appreciable, while the change from the third to the ninth hour was less than that which occurred in unbuffered broth. The greatest change occurred during the period of maximum utilization of dextrose and acid production, that is, from the sixth to the ninth hours.

The final hydrogen-ion concentration was not reached in twelve hours as a rule, a drop from 0.1 to 1.2 pH being noted between the twelfth and twenty-fourth hours. The final hydrogen-ion concentration reached was lower than in unbuffered broth. A larger number of organisms were viable in seventy-two hours, and there was a greater degree of alkaline reversion in buffered broth. In many cases this alkaline reversion was accompanied by an increase in titratable acidity. This might be explained by the union of basic substances with the acids to form an alkaline-reacting compound. This would be accompanied by a decrease in free hydrogen-ions, but an increase in titratable acidity. This alkaline reversion with an increase in titratable acidity was especially marked in the case

of strain No. 5. This organism also remained viable over a longer period of time than the other organisms. No significant individual differences were noted, and there was no parallelism between the metabolic changes and antigenic differences.

(c) *Growth Rates.*

Tables XIX and XX show the growth rates of the organisms under the conditions given above. Buchanan (1918) divided the growth period of an organism into seven phases, namely: First, initial stationary period; second, lag phase; third, maximum or logarithmic increase; fourth, decreasing rate of multiplication; fifth, maximum stationary period; sixth, accelerated death; seventh, death at a constant rate. Chesney (1916) divided the growth period into four phases, namely: First, initial stationary period; second, lag, or growth at slowly increasing rate; third, maximum period; fourth, decreasing rate.

The lag phase has occupied the attention of several investigators. Rahn (1906) noted that the greater the inoculum the shorter the lag. He was not able to get rid of the lag phase entirely, however. Müller (1896) noted that with *B. typhosus* the lag varied with the age of the inoculum and considered that it was due to the injury suffered by the bacteria in the previous medium. Barber (1908) was able to eliminate the lag phase entirely by transferring organisms during the period of logarithmic increase.

If one follows Chesney (1916), an examination of my tables shows that when the first determination was made three hours after inoculation, the stationary period did not appear. The lag phase occurred in the unbuffered broth during the first six hours as a rule, though growth at an increasing rate usually occurred from the third to the sixth hours. The period of maximum growth occurred from the sixth to the ninth hour, while from the ninth to the twelfth hour the organisms were multiplying at a decreasing rate. As no consistently satisfactory counts were obtained on 24-hour samples, the last phases do not appear in the tables. It may be seen, then, that the growth rate calculated in logarithms paralleled dextrose utilization and acid production.

In buffered broth the three-hour periods used were usually too few to distinguish sharply between the lag phase and growth at increasing rate. It is evident, however, that the period of maximum growth occurred between the sixth and ninth hours. The amount of growth taking place between the sixth and ninth hours was

greater in buffered than in unbuffered broth. From the ninth hour, the same increase at diminishing rate was observed as in the unbuffered broth.

The increase in the number of organisms was greater during the period of logarithmic increase in the buffered broth than in the unbuffered, but the levels reached were seldom higher in the buffered broth. There was a tendency for the depression in growth rate to occur earlier in the unbuffered than in the buffered broth.

During the period of maximum growth, from the sixth to the ninth hour, the hydrogen-ion concentration average was from pH 6.7 to pH 5.5 in unbuffered broth and from pH 7.1 to pH 6.3 in buffered broth. One might expect, therefore, to find inhibition beginning before the ninth hour in unbuffered broth. Inhibition was noted in both buffered and unbuffered broth at the ninth hour, being somewhat more marked in the former. This would indicate that factors other than the hydrogen-ion concentration were concerned.

(*d*) *Rate and Relation of the Utilization of Dextrose to the Final Hydrogen-ion Concentration and Titratable Acidity, Inhibition and Death.*

A comparison of the utilization of dextrose, titratable acidity, change in hydrogen-ion concentration and growth rates calculated as logarithms of the actual number of organisms in buffered and unbuffered broth shows that in general, they tend to parallel each other. There are certain differences, however, that may be noted. The sugar utilization, titratable acidity and growth rates were much more nearly parallel than were these changes with the change in hydrogen-ion concentration. The buffer effect of the medium seemed to suppress the change in hydrogen-ion concentration, so that in spite of the utilization of dextrose and the accumulation of acids there was very slight change in the hydrogen-ion concentration. The inhibition beginning at the ninth hour also would not be apparent if hydrogen-ion concentration determinations only were done. In unbuffered broth the final hydrogen-ion concentration was reached by the twelfth hour, but there was considerable utilization of dextrose and accumulation of acids as long as the organisms were viable. In buffered broth there was an appreciable change in hydrogen-ion concentration from the twelfth to the twenty-fourth hour, but this change did not give one an idea of the amount of dextrose used.

It was also noted that although the amount of dextrose used and

the growth rate in logarithms were roughly parallel in both buffered and unbuffered broth, the amount of dextrose used per organism was much greater during the first three hours than at any subsequent time. After the third hour the amount used per organism decreased steadily until between the ninth and twelfth hours the amount of dextrose used per organism was inappreciable. Table XXI brings out these facts clearly. It is realized that these figures are approximate rather than exact. Determinations at shorter intervals of time would be necessary to determine exactly the amount used per organism. However, we feel that these figures are as exact as the common use of logarithms to indicate the actual number of organisms present. It seems to be sufficiently exact to indicate the striking difference between the amounts of sugar used per organism at different times during the twelve-hour period.

TABLE XXI.—Utilization of dextrose per organism.

BROTH.	Hours.	Dextrose used in grams.	Dextrose used per organism.
Unbuffered	3	.01	.000,000,08
	6	.10	.000,000,000,5
	9	.18	.000,000,000,6
	12	.27	.000,000,000,2
Buffered	3	.07	.000,000,056
	6	.10	.000,000,032
	9	.32	.000,000,000,050
	12	.68	.000.000.000,013

The organisms in unbuffered broth used the largest amount of dextrose per organism during the first three hours. The amount used during the next three hours was one one-thousandth of that used during the first three. From that time on, there was very little dextrose used per organism although the maximum rate of reproduction usually started about the sixth hour.

In buffered broth during the first three hours there was a maximum utilization of dextrose per organism. During the next three hours there was only a slight decrease in utilization per organism. Between the sixth and ninth hour there was noted the same enormous drop in the amount of dextrose used per organism despite the fact that here, too, maximum reproduction of the organisms was initiated at the sixth hour. The utilization of dextrose per or-

ganism in buffered broth was much less than in unbuffered broth from the sixth to the twelfth hour. It will be noted also that although the total amount of dextrose used in buffered broth was much greater than in unbuffered, the actual increase in numbers of organisms was about the same. This evidently indicates that in buffered broth each organism was capable of metabolising more sugar than in unbuffered broth.

DISCUSSION.

A discussion of the foregoing results brings up the following questions:

First, the amount of dextrose which may be used by an organism and the influence of the medium upon this utilization.

Second, a comparison of the utilization of dextrose, change in titratable acid, hydrogen-ion concentration and growth rate.

Third, the influence of the products of dextrose metabolism on the activity of the organisms.

Fourth, constancy of the changes exhibited by *B. typhosus* and a comparison with other organisms.

Comparatively few authors have followed the quantitative utilization of dextrose hour by hour, though many have determined the minimum amount of dextrose necessary to give the final hydrogen-ion concentration.

Duchacek (1904 *a* and *b*), using peptone water plus 1 per cent dextrose, found that *B. typhosus* used 18 per cent of the dextrose in eleven days and 41 per cent in thirty days. The total acidity was calculated by adding together the different fractions of the acids determined by chemical analysis. He noted that the total amount of acid as determined by titration was more than can be accounted for by the acids found. It is difficult to see why there should be so much sugar used between the eleventh and thirtieth days since in my experiments and those of other observers on the colon-typhoid group the organisms were seldom viable after ninety-six hours in dextrose broth.

Cole and Onslow (1916) state that *B. typhosus* gave an alkaline reversion with 0.15 per cent dextrose but remained permanently acid in 0.2 per cent dextrose. That this would be true only in a medium low in buffer content may be seen from my tables. Sears (1917) found that *B. typhosus* used 0.138 to 0.270 gram of dextrose on the first day in broth and 0.399 to 0.487 gram on the eighth day. Here again, the effect of an abundant buffer may be noted. This result

agrees with mine except that usually more sugar was used during the first twenty-four hours.

Kligler (1916) using ammonia production as an index of protein metabolism, noted that no ammonia was produced by *B. typhosus* in five to seven days where 0.5 per cent of dextrose was present. The effect of buffers was noted, and he found that where a buffer was present the final concentration of hydrogen-ions in the medium was lower. *B. typhosus* reached pH 4.6 to 4.8 in unbuffered peptone water and pH 4.8 to 5.1 in peptone water buffered with phosphates. This is in accord with my results. My results in buffered and unbuffered broth are in entire agreement with Clark (1915*a*), Bunker (1919), Burton and Rettger (1917), Schoenholz and Meyer (1919), Bronfenbrenner (1918*b*) and Salter (1919), who found in general that the presence of buffers stimulates growth, prolongs the life of the organisms and greatly increases the amount of dextrose used. The results obtained in my experiments indicate that the amount of dextrose used by *B. typhosus* was so dependent upon the buffer content of the medium that it would always be necessary to know the kind of medium used and its buffer content before any comparison could be made with the work of other authors on *B. typhosus* or other organisms.

A number of authors have assumed that the dextrose utilization, change in titratable acidity, growth rates, and change in hydrogen-ion concentration, parallel each other so closely that by following the change in hydrogen-ion concentration, the other changes mentioned will be sufficiently revealed.

Kligler (1916) states that the hydrogen-ion concentration serves to show the amount of sugar which an organism can use without sufficient acid to limit growth and also the rate at which the sugar is utilized. Foster (1921*b*), Anderson (1920), Cullen and Chesney (1918) and Lord and Nye (1919) assume that the above factors are parallel.

In my results the utilization of dextrose and increase in titratable acidity paralleled each other very closely. The lag period, period of maximum increase in number of organisms and period of decrease coincided almost exactly with the utilization of dextrose and increase in titratable acidity when the growth rates were followed by means of the logarithms of the actual number of organisms. However, if the dextrose used per organism was calculated it will be seen that the amount used was very much greater during the lag period than

during the period of maximum reproduction. It might be suggested that this could be due to a storing up of the dextrose in some form in the bodies of the bacteria for the purpose of release during the time of active reproduction. This might be effected by an enzyme. This suggestion is borne out by the smaller accumulation of acid during this period than during the period of maximum growth. In comparing the amount of dextrose used per organism in buffered and unbuffered broth, it will be seen that much more dextrose per organism was used during the lag period in buffered broth. This might be due to the stimulating effect of the buffer on the storage of the carbohydrate in each organism or the lack of buffer might decrease the enzyme causing the storage.

The changes in hydrogen-ion concentration do not follow the dextrose utilization as closely as does the change in titratable acidity, since during the first three hours there was no apparent change in hydrogen-ion concentration. The titration of acids, or the quantitative determination of sugar shows that an appreciable change has taken place. Since little or no change takes place in hydrogen-ion concentration after the final concentration is reached, it might be assumed that no changes occurred. That this is not the case is shown by the above determinations. It is not safe, therefore, to assume, as Chesney (1916) does that because there is no change in hydrogen-ion concentration there is also no metabolic activity going on.

DeBord (1923) using *B. subtilis, B. pyocyaneus, B. coli Cl. botulinum* and *Cl. sporogens,* found that sugar utilization and change in hydrogen-ion concentration paralleled each other closely in those organisms which fermented dextrose with acid production. *B. subtilis* and *B. pyocyaneus,* however, showed increasing alkalinity in spite of utilization of the sugar.

Foster (1921*b*) has divided the change in hydrogen-ion concentration produced by streptococci into five periods, namely: First, stationary period; second, lag or acid at increasing rate; third, acid at maximum or logarithmic rate; fourth, acid at decreasing rate; fifth, stationary period. The changes in hydrogen-ion concentration observed by me would also fall into these five periods, but in dextrose and titratable acid determinations the first period was not noted. The fourth period, acid at decreasing rate, continued from the ninth to the twenty-fourth or forty-eighth hour. The fifth period did not appear as long as any organisms were viable.

I have found, in agreement with most observers, that the period of

logarithmic increase and the maximum utilization of dextrose are parallel. Jones (1920), using pneumococci, showed that the maximum period of growth occurred near neutrality, but that sugar utilization was greatest (as indicated by the change in hydrogen-ion concentration) after the maximum period was ended, that is, during the phase of negative acceleration.

Cohen and Clark (1919) noted the same phenomenon with *B. coli* and suggested that the effect of acidity on fermentation and on growth are independent. My results indicated the reverse of this, since the maximum amount of dextrose used per organism was greatest before the initiation of the period of maximum increase.

It is generally assumed that the maximum utilization of dextrose takes place during the period of logarithmic increase, and this appears to be the logical assumption, since organisms are generally believed to use dextrose for the production of energy. It would appear from my findings that the organisms stored up energy by the utilization of dextrose and then used this stored energy during the period of most rapid multiplication, instead of breaking down the dextrose available in the medium. The apparent parallelism between the period of maximum utilization of dextrose as a whole and the period of logarithmic increase is due to the very large number of organisms present during this period, each one of which is using a very slight amount of dextrose.

Recently it has been assumed that the inhibition and death of organisms in a medium containing a utilizable carbohydrate was largely due to the effect of the hydrogen-ions. There can be no doubt that they play a prominent role. Cohen and Clark (1919), however, noting the early inhibition of the growth of *B. coli* in plain broth where there was no appreciable change in hydrogen-ion concentration, warn against laying too much stress on the toxic effects of the hydrogen-ions. My results show that in unbuffered broth inhibition took place at the ninth hour with an average pH of 5.5. In buffered broth the same or sometimes even greater degree of inhibition took place with a pH of 6.3. It would seem from these results that the great accumulation of acids and other products of metabolism, as well as the hydrogen-ion concentration, exerted an effect on the growth of the organisms.

The constancy of the utilization of dextrose and the other factors here discussed for the strains of *B. typhosus* used has been remarkable. No constant differences between the typical strains of *B. typhosus* were noted with the exception of No. 5. There was no cor-

relation between the serological differences and dextrose metabolism. It is practically impossible to compare the results given here with those of other authors because of the variety of the media used. It would seem, however, that streptococci use less sugar than *B. typhosus*, *B. dysenteriæ* about the same, the paratyphoids and *B. coli* slightly more and *B. aerogenes* and *B. cloacæ* decidedly more than *B. typhosus*.

SUMMARY.

The following gives a summary of the results obtained:

1. The utilization of dextrose was largely dependent on the buffer content of the medium. Buffers stimulated the utilization of dextrose per organism to a marked degree, but the total number of organisms was little if any greater in buffered than in unbuffered broth.

2. The utilization of dextrose appeared to be a function of living cells. The maximum dextrose utilization per organism took place during the lag period. This was followed by the period of maximum growth, during which little dextrose per organism was used. The increase in titratable acid coincided in quantity and rate with the utilization of dextrose.

3. The change in hydrogen-ion concentration did not serve as an accurate indicator for the amount of dextrose used, since there was little or no change during the first six hours of growth, although an appreciable amount of dextrose had been used with a corresponding accumulation of acids. The hydrogen-ion concentration remained stationary after the final concentration was reached while dextrose utilization continued as long as the organisms were viable.

4. The titratable acidity gave a more accurate indication of the amount of dextrose used than did the change in hydrogen-ion concentration.

5. Alkaline reversion to a slight degree after the final hydrogen-ion concentration was reached did not signify complete utilization of the dextrose.

6. Death occurred partly as a result of the toxic effects of the free hydrogen-ions and partly from the accumulation of other toxic growth products.

7. The variations between the strains used were not of any significance except in the case of No. 5, and could not be correlated with antigenic differences.

DISCUSSION OF IRREGULAR STRAINS.

Before proceeding to a discussion of the results here presented, it might be of interest to note the irregularities observed in certain strains of the organisms used. Strains Nos. 21, 40, and 12 (Rawlings) were apparently typical strains of *B. typhosus*, serologically, reacting in high titre with the various sera used, and all belonging to one group as shown in Part I. These strains fermented xylose slowly. Nos. 21 and 12 gave acid after fourteen to twenty-one days. No. 40 gave a very slight amount of acid, pH 5.8 after three weeks. All of these organisms gave daughter colonies on xylose eosin-methylene-blue plates but it was not possible to select rapid fermenters from those colonies. These organisms have been grown in the presence of xylose on plates and in xylose broth for six weeks without acquiring the ability to ferment xylose more rapidly. No. 12 produced a small amount of acid in one hundred and twenty hours, but on transferring to fresh broth it produced no acid until the seventh day.

In arabinose, raffinose, dulcite and rhamnose these organisms never produced acid. Daughter colonies were observed in the case of No. 40 in arabinose, none were formed in dulcite and all formed daughter colonies in rhamnose. No fermenting strains could be picked from these colonies.

No. 5 was an organism which exhibited some of the cultural irregularities noted for the typical strains of *B. typhosus* considered above, and in addition it appeared to be entirely inagglutinable. In an effort to identify it, many different antityphoid sera have been used with entirely negative results. *B. paratyphosus B*, *B. paratyphosus A*, *B. pullorum* and *B. sanguinarium* antisera have been used with equally negative results. No drop in titre for the homologous organisms resulted when the above antisera were absorbed by No. 5. Antiserum prepared by the injection of No. 5 into rabbits agglutinated No. 5, but did not agglutinate any of the typical strains of *B. typhosus* nor *B. paratyphosus A* or *B*, *B. Flexner* or *Shiga dysenteriæ*. Cultures picked from separate colonies at different times have never yielded agglutinable strains. The serum of the patient from whom this organism was isolated did not give a positive Widal. The cultural reactions of No. 5 were also somewhat irregular in the following respects: there was slow but definite blackening of lead-acetate agar; a rapid return to alkaline in litmus milk; fermentation in rhamnose and no fermentation in xylose. In twenty-four hours the rhamnose eosin-methylene-blue plates were negative, in forty-

eight hours the primary colonies showed small but dark daughter colonies, and in seventy-two hours all the colonies were quite dark with a greenish sheen. It was not possible to select daughter colonies producing typical acid colonies in twenty-four hours. In xylose eosin-methylene-blue plates in seventy-two hours, faintly dark daughter colonies were visible. Organisms picked from these daughter colonies did not ferment xylose. Growth for six weeks in xylose broth did not enchance the power of this organism to ferment xylose. In all cases, the reaction was alkaline in two per cent xylose broth during a period of twenty-five days.

This organism also differed in the rate and quantity of dextrose used. Repeated tests showed that it reached pH 5.0 to 5.3 in twenty-four hours, at the end of a week it was viable and still using sugar, but an alkaline reversion had taken place which reached pH 5.5. During the second week, the hydrogen-ion concentration remained practically stationary and very minute amounts of sugar were used. The organisms were frequently dead at the end of the second week. This organism, then, showed a type of sugar metabolism which resembled *B. ærogenes* or *Morgan's bacillus,* since it continued to use dextrose in spite of an alkaline reversion.

No. 5 also had a wider range of growth than the other organisms studied, being viable at pH 9.6. In plain broth adjusted at pH 5.0 the broth was sterile in twenty-four hours, in one per cent dextrose broth, however, although a reaction of pH 5.0 was often reached in twenty-four hours, the organism continued to live and within forty-eight hours was beginning to give an alkaline reaction in the culture medium.

The identification of this organism involved many considerations. The ability of an organism to gain or lose fermentative powers or antigenic properties by natural or artificial means involved the question of the reliability of fermentation and antigenic reactions and also the question of mutation in bacteria. A survey of the literature bearing on these questions revealed many conflicting opinions. Winslow *et al.* (1919), after a comprehensive review of the variations observed in fermentation reactions, reach the conclusion that in the great mass of colon-typhoid strains cultivated under standard conditions the fermentative characters exhibit a high degree of correlation with other biochemical, serological and pathogenic properties. The fermentation or nonfermentation of lactose, sucrose and xylose seem to be especially important and stable characters. Krumwiede (1918), on the other hand, states that variations in physiological

function of bacteria occur with frequency, while general experience indicates a high degree of stability as regards the antigenic properties.

Many variations both spontaneous and induced are recorded in the literature. Thro (1914) concluded that carbohydrate fermentation could not be used to separate strains of streptococci since different colonies picked from a plate streaked from a single colony gave great variations in acid production. Broadhurst (1915) observed spontaneous variations in the direction of the fermentation of carbohydrates not previously fermented. The carbohydrates which showed the greatest variation were those less frequently fermented by streptococci, namely mannite and inulin. Glenn (1911) by plating was unable to select colonies which gave large and small amounts of acid uniformly. Raubitschek (1913) observed variation in acid production, using strains isolated from the different organs of a typhoid cadaver, basing his conclusions on "strong red" and "weak red" reactions in litmus broth medium.

Many authors have reported on the variations produced by artificial means. The best example of this is probably the work of Twort (1907) who induced lactose fermentation in a strain of *B. typhosus* by long cultivation in the presence of lactose. Penfold (1911, 1912) confirmed and extended Twort's work. He reported that lactose-fermenting strains threw off nonfermenting mutants. Many of the fermenting and nonfermenting mutants were picked from daughter colonies. Muller (1911) had observed daughter colonies on rhamnose, but he apparently did not observe acid formation in these colonies, as he suggested that they might be organisms which were capable of using this pentose without acid formation. Teague (1920), Teague and Morishima (1921) and Morishima (1921), reported acid formation in daughter colonies in xylose with strains of *B. typhosus* which fermented this pentose slowly, and also in rhamnose, raffinose and arabinose. In the last-mentioned carbohydrates there was no acquisition of new fermentative power on transfer into broth. The slow xylose-fermenters could be changed into rapid fermenters by this means, however. There was no correlation between xylose fermentation and serological reactions. Teague (1920) concluded that these irregular fermentations were not evidence against the homogeneity of *B. typhosus*.

The occurrence of spontaneous variation in agglutinability in *B. typhosus* has been reported by Weiss (1917), Mock (1919), Durham (1901), Burri and Dugeli (1919), Raubitschek (1913), Toyoda

(1922), and others. Recently several authors have tried to induce antigenic variations by various means. Gardner and Walker (1921) by plating were able to select colonies which varied in antigenic properties. These variations were not stable, however. Arkwright (1921) considered that any culture may contain strains varying in their agglutinogenic powers. The temporary preponderance of one or the other agglutinogenic property in the mass of the population of a particular culture determines the serological character of that culture. Jordan (1926) has reported the appearance of two antigenically dissimilar strains from a single cell line. The antigenic peculiarities were associated with the formation of rough and smooth colonies on agar plates. Blankenhorn *et al.* (1923) reported the isolation of an atypical *B. typhosus* from a periosteal abscess. The patient's serum agglutinated the isolated organism in dilutions of one to eight but did not agglutinate stock cultures of *B. paratyphosus A*, B or *B. typhosus*. Serum prepared from this organism agglutinated the Rawlings strain of *B. typhosus* in low dilutions and slowly. It was also irregular in its cultural reactions in that it did not ferment mannite nor dextrin. It produced slight acid in xylose. Lead acetate was not mentioned. They considered that this organism was probably influenced by its long existence in the body of the patient.

Benians (1920) produced variation in *B. dysenteriæ* by growth in a gum tragacanth abscess in a guinea pig. Agglutinable and inagglutinable strains could be picked according to the different colonies observed after plating the material from the abscess. Ecker (1923) was able to induce similar changes in the Rawlins strain of *B. typhosus* by the injection of this organism into a sterile gum tragacanth abscess in a guinea pig. Simonds (1915) by growing *B. typhosus* in dextrose broth over long periods of time was able to produce a variant of *B. typhosus* which had greatly enhanced agglutinogenic powers.

Summarizing my results in light of the foregoing review, the following facts are apparent: Strains Nos. 12, 21 and 40 were typical in their serological reactions but were irregular in xylose fermentation. They fermented xylose slowly and could not be trained to give rapid fermentation although they produced daughter colonies on xylose plates. Strain No. 5 did not ferment xylose but did ferment rhamnose. It also differed from typical strains of *B. typhosus* in its dextrose metabolism. It was not agglutinated by the patient's serum nor by antityphoid serum. It does not, however, differ any more

radically from typical strains of *B. typhosus* than do those reported by Ecker (1923), Blankenhorn (1923), Berridge (1920) and others. Krumweide,* in spite of his belief in the stability of antigenic properties falls back on rhamnose fermentation and classes this organism as a paratyphoid strain because of its fermentation of rhamnose. No. 5 was an organism which had no apparent antigenic relation with any other organism of the colon-typhoid group. It had the property of attacking dextrose and proteins simultaneously. In this respect it resembled the colon, para-colon and dysentery organisms. Its resemblances to *B. typhosus* have been summarized above. In its action on rhamnose and xylose, it resembled the paratyphoid group. Since it failed to conform to either the cultural or serological criteria but was irregular in both, it would seem wiser to consider it as an unidentified paralike organism which does not produce gas.

GENERAL DISCUSSION.

The question of the criteria to be used in the identification of typhoid-like organisms and the homogeneity of the *B. typhosus* group has been raised by the work reported here.

The classification of bacteria offers peculiar difficulties since morphological variations are so slight that physiological and antigenic characters must be used. Winslow (1906*a*) points out that in unicellular organisms acquired characters are propably inherited to a higher degree than with other forms, and therefore they are more influenced by environmental conditions. Four methods for the classification of bacteria may be used: First, morphological; second, cultural; third, antigenic; fourth, a combination of the preceding. It is fairly well accepted now that a purely morphological classification like that of Migula is entirely insufficient. Chester (1909), Andrews and Horder (1906), Winslow (1906), and, more recently, Bergey (1923) in his manual have used the cultural method of classification or a combination of cultural and morphological methods. Krumwiede (1918) and others consider the antigenic properties of bacterial protoplasm as much more stable than the physiological reactions, and would therefore place more confidence in the antigenic characters for classification and identification. Jordan (1920) recognizing the correlation between antigenic and cultural characters, would use both for identification.

A survey of the literature bearing on the cultural and antigenic

* Information given in a letter from Dr. Krumweide after he had examined this organism.

characters used in the classification of the different groups of organisms reveals the fact that homogeneity for any one group is the exception rather than the rule. Streptococci have been classified by fermentation reactions by Gordon (1905), Andrews and Horder (1906), Holman (1916), Blake (1917), and others. The concensus of opinion seems to be that the fermentation of certain carbohydrates is remarkably constant. The antigenic classification of the streptococci has proved difficult if not impossible because of their antigenic heterogeneity. *Streptococcus scarletinæ* may be an exception in that it is apparently quite homogeneous in its antigenic properties as shown by the work of Dochez (1924) and Dick (1924). With only a few exceptions it has been impossible to correlate fermentation and antigenic characters among the streptococci. It is generally accepted now that the classifications such as Brown's (1919), Blake's (1917), and Holman's (1916), based entirely on physiological reactions, offer the best solution of the problem.

Pneumococci exhibit quite constant physiological reactions as a group, but the work of Dochez (1913), Gillispie (1916), Neufeld (1910), and others, have demonstrated distinct and constant antigenic differences within this group.

Meningococci have been divided into at least four groups which are not as well defined as those of pneumococci.

Torrey and Buchell (1922) have shown that gonococci cannot be divided into clear-cut immunological types, but they suggest a classification under three headings: (*a*) Regular; (*b*) intermediate; and (*c*) irregular strains. They comment on the marked tendency to antigenic lability on the part of the gonococcus and believe that instability of antigenic constitution is a characteristic of this group.

B. diphtheriæ have been cited as being so homogenous as to constitute a true genus. Recently, however, antigenic differences have been demonstrated by agglutination and complement-fixation reactions which subdivide this group into at least two groups. These antigenic differences are not correlated with cultural differences, and it is interesting that there is slight if any correlation between the antigenic differences and toxin production. That is to say, antitoxin prepared from one strain will neutralize toxin from the other strains. There seems to be a difference here between physiological, antigenic and functional characters of the organism.

Clear cut antigenic differences are present among stains of *B. dysenteriæ* and in this case cultural and antigenic differences are closely correlated.

The paratyphoid group has been peculiarly difficult to classify because of the lack of correlation between cultural and agglutination reactions. Sherwood, Downs and McNaught (1920) have observed antigenic differences in organisms resembling *B. enteritidis.* Zinsser (1923) remarked that we should be slow to assume that either agglutination or culture similarity are to be regarded as definitely signifying biological relationship.

Jordan (1918) emphasizes the fact that variations in both fermentation and agglutination are common in the paratyphoid-enteritidis group. The most permanent characters of the group seem to be the fermentation of rhamnose by *B. paratyphosus,* but not by *B. typhosus;* the fermentation of xylose by *B. typhosus,* but not by *B. paratyphosus A.* He considers the above reactions more important than the agglutination reactions.

B. typhosus has long been considered the prototype of an organism in which the protoplasm is antigenically homogeneous. If such is the case, it seems to offer an exception to the general biological rule as brought out by the foregoing survey.

It is also difficult to dismiss as unimportant the numerous reports of definite antigenic differences. It is true that a careful examination of these reports and of my results, as shown in Part I, shows that the differences are apt to be quantitative rather than qualitative. An organism which agglutinates weakly or not at all with antityphoid serum may absorb agglutinins to an appreciable extent or it may produce an antiserum which agglutinates typical strains of *B. typhosus* in high dilution. That it is impossible to use the agglutination or even the absorption reaction as an absolute criterion for identification of *B. typhosus* strains, is indicated by the interagglutination and absorption reactions between *B. typhosus* and *B. pullorum* and *B. sanguinarium.* Here there is a marked antigenic similarity and somwhat less marked cultural similarity between organisms entirely unrelated as to source and pathogenicity.

The occurrence of agglutinative and cultural irregularities in certain epidemics of typhoid fever point to a possible sanitary significance of different strains of *B. typhosus.* Many suggestions have been offered as to the advisability of using more than one strain in typhoid vaccines. My results point to the occurrence of such distinct and constant antigenic differences between certain strains of *B. typhosus* that the use of a polyvalent vaccine would be advisable.

It would seem wiser in view of the foregoing reports and the re-

sults of my experiments to use a combination of cultural and antigenic characters for identification of organisms.

The utility of any reaction for identification purposes involves the following factors: First, the constancy of the reaction under stated conditions; second, spontaneous variations in the reactions; third, induced variations.

The apparent constancy of cultural reactions depends to a marked degree on the medium used, the amount and age of inoculum, the time and temperature at which the experiment is carried out.

My results show that the buffer content of the medium exerts a profound effect on sugar metabolism. With a vigorously growing organism it would be entirely possible to use up all the fermentable carbohydrate present and show an alkaline reaction in twenty-four hours if insufficient carbohydrate was used.

The effect of the presence of a fermentable carbohydrate in protein metabolism has been clearly shown by Kendall (1916), Sears (1917), DeBord (1923), and others. In using cultural reactions for identification, it is necessary, therefore, to standardize the culture medium and the tests used to indicate the various reactions taking place.

It seems possible that the constancy of antigenic properties might also be influenced by the culture medium on which the organisms are grown. Simonds (1915) has shown that continuous growth in the presence of dextrose influenced the antigenic properties of a certain strain of *B. typhosus*.

Salts, hydrogen-ions and colloids in the suspension of organisms used and in the antiserum have a great influence on the demonstration of antibody reaction in the agglutination and absorption reactions as well as in other immunological reactions.

Here again a standardization of these variable factors would be desirable and would make it possible to compare the results obtained by different authors.

The occurrence of spontaneous and induced variations in bacteria in both cultural and antigenic reaction has frequently been noted Berridge and Glynn (1920), Broadhurst (1915), and others, have noted the spontaneous acquisition or loss of fermentative characters in a number of organisms. The spontaneous acquisition of agglutinability by strains which are inagglutinable when first isolated is well known. Toyoda (1922), Mock (1919), Benians (1920), and others, have reported on sponetaneous changes in agglutinability in cultures of *B. typhosus*.

Enhanced fermentation reactions have been reported by Twort (1907), Penfoed (1912), Muller (1919), D'Aunoy (1913), and many others, by the continuous growth of an organism in the presence of carbohydrates. My results on this point have been entirely negative. Dawson (1919) has been able to induce variation in both cultural and antigenic properties in *B. coli* by growth on various media. Bergstrand (1923), Berridge and Glynn (1920), and Jordan (1926), have reported the occurrence of antigenic variability in organisms picked from different colonies of pure cultures of *B. coli, B. dysenteriæ* and *B. paratyphosus B.*

Ecker (1923), Blankenhorn (1923), Gay (1913), Meyer (1921), and others, have obtained changes in antigenic properties in *B. typhosus* by animal inoculation.

It must be recognized that it is only occasionally that the spontaneous and induced variations noted above have any practical bearing on the identification of organisms. However, whenever an organism is to be identified the possibilities of such irregularities must be considered. There seems to be meager evidence as to the stability of these variations. The antigenic and cultural variations reported by me have remained unchanged for three years.

In summing up the criteria to be used in the identification of organisms, the following factors should be considered.

1. Physiological reactions, including (*a*) morphological and staining reactions, (*b*) cultural reactions, both fermentation of carbohydrates and protein utilization.

2. Antigenic reactions, including agglutination and absorption tests, and in some cases complement fixation, toxin production and pathogenicity.

3. A correlation of the above factors and a consideration of the significance of any variations noted.

In the results reported here I found the strains of *B. typhosus* used to be homogeneous in regard to the cultural reactions with the exception of three strains which fermented xylose slowly and one strain which gave a rapid return to alkaline in litmus milk. These strains were much less homogeneous antigenically than they were culturally. It would seem from my results and the result of others that *B. typhosus* is less homogeneous in its antigenic properties than is commonly supposed. It, therefore, conforms to what seems to be a general biological law among bacteria, namely, that organisms within the same group exhibit antigenic and cultural variations, and that these variations are not necessarily correlated.

GENERAL SUMMARY AND CONCLUSIONS.

The following conclusions were drawn from the study of thirty-one strains of *B. typhosus*. One unidentified organism, No. 5, was considered separately:

1. Qualitative cultural reactions were constant in glucose, mannite, maltose, sucrose, lactose and rhamnose. Irregularities in dextrin and salicin fermentation and litmus milk were noted. Several strains fermented xylose slowly. These irregularities could not be correlated with the age of the cultures nor the source. No variation occurred in indol reaction, gelatin liquefaction, nor lead-acetate reaction.

2. Antigenic irregularities as demonstrated by agglutination, absorption and Widal tests occurred in these strains of *B. typhosus*. These irregularities were constant, but were quantitative rather than qualitative. The strains used may be divided into three groups on the basis of these reactions.

3. These antigenic irregularities were sufficiently constant to make advisable the use of several strains for Widals and also possibly for typhoid vaccines.

4. The growth range of *B. typhosus* was found to be pH 5.0 to pH 9.0, the optimum range, from pH 6.0 to pH 7.5.

5. There was a slight increase in titratable acidity in broth to which no carbohydrate had been added due to the presence of muscle sugar. The hydrogen-ion concentration and titratable acid after twenty-four hours became increasingly alkaline.

6. The metabolism of these strains of *B. typhosus* in broth containing various carbohydrates was remarkably constant. The final hydrogen-ion concentration reached was characteristic for each carbohydrate with the strains used.

7. The final hydrogen-ion concentration was usually reached in twenty-four hours.

8. A definite amount of dextrose was necessary to enable the organisms to reach their final hydrogen-ion concentration, and the amounts varied with the buffer content of the medium.

9. The initial hydrogen-ion concentration had little effect on the metabolism of the organisms, provided it was within the optimum range of growth.

10. The viability of the organisms was determined by the free hydrogen-ions produced by fermentation and by the kind of carbohydrate fermented.

11. Slight but distinct alkaline reversion occurred after the final hydrogen-ion concentration was reached. The lower hydrogen-ion concentrations given in less available carbohydrates was correlated with a greater degree of alkaline reversion. This slight alkaline reversion did not signify complete utilization of the carbohydrate.

12. The amount of dextrose used and the acid formed was constant for all the strains under stated conditions, that is to say, the composition of the medium determined the amount of dextrose used and the accumulation of acids. The presence of buffers stimulated the metabolism of dextrose by the bacteria much more than it stimulated growth.

13. The amount of dextrose used and titratable acid paralleled each other closely for seventy-two hours. The change in hydrogen-ion concentration lagged behind the accumulation of acids and the utilization of dextrose so that it would be impossible to use the change in hydrogen-ion concentration as an accurate index of the amount of dextrose used, the change in titratable acid or growth rates.

14. Titratable acidity offered a more complete indication of the amount of dextrose used than did the change in hydrogen-ion concentration.

15. The period of maximum dextrose utilization per organism preceded the period of maximum reproduction. The period of logarithmic increase coincided, however, with the period of the greatest total decrease in dextrose.

16. It was impossible to correlate the antigenic differences noted in Part I with any peculiarities in cultural or metabolic reactions.

17. An unidentified organism was described—No. 5. It was inagglutinable in all sera used. It resembled *B. typhosus* in its cultural reactions except that it did not ferment xylose and did ferment rhamnose. The metabolism of this organism in dextrose resembled that of *B. dysentery* or *B. coli* in that simultaneous acid and alkaline fermentation seemed to occur. It seemed to be a new organism which might ultimately be classified as a member of the dysenteriæ group or as a non-gas-producing paratyphoid.

BIBLIOGRAPHY.

1. ANDREWS, F. W., and HORDER, T. J. 1906. Lancet, 2-708.
2. ANDERSON, R. A., and SCHULTZ, O. T. 1920. Jour. Exper. Med., 33-653.
3. ARKWRIGHT, J. A. 1921. Jour. Path. and Bact., 24-36.
4. D'AUNOY, R., 1923. Jour. Infect. Dis., 33-391.
5. AYERS, S. H., and MUDGE, C. 1922. Jour. Infect. Dis., 31-40.
6. AYERS, S. H., and RUPP, P. 1922. Jour. Infect. Dis., 30-388.
7. AYERS, S. H., JOHNSON, W. T., and DAVIS, B. J. 1918. Jour. Infect. Dis., 23-290.
8. AYERS, S. H., and RUPP, P. 1918 *a*. Jour. Infect. Dis., 23-188.
9. AYERS, S. H., and RUPP, P. 1918 *b*. Jour. Infect. Dis., 23-208.
10. AVERY, O. T., and CULLEN, G. E. 1919 *a*. Jour. Expr. Med., 29-215.
11. AVERY, O. T., and CULLEN, G. E. 1919 *b*. Jour. Expr. Med., 30-359.
12. AVERY, O. T., and CULLEN, G. E. 1918. Jour. Exp. Med., 29-369.
13. BARBER, M. A. 1908. Jour. Infect. Dis., 5-379.
14. BENIANS, T. H. C. 1920. Jour. Path. and Bact. 23-171.
15. BENGSTEN, IDA A. 1919. Jour. Infect. Dis., 24-428.
16. BERRIDGE, E. M., and GLYNN, ERNEST. 1920. Jour. Path. and Bact., 23-199.
17. BERMAN, H., and RETTGER, L. F. 1918. Jour. Bact. III, 367.
18. BERMAN, H., and RETTGER, L. F. 1918 *b*. Jour. Bact. III, 389.
19. BERGEY, D. H. Manual of Determinative Bact. 1923. (Williams & Wilkins.)
20. BLAKE, F. G. 1917. Jour. Med. Res., 36-99.
21. BLANKENHORN, M. A., ECKER, E. E., and KING, M. K. 1923. Jour. Infect. Dis., 32-95.
22. BROADHURST, JEAN. 1913. Jour. Infect. Dis., 13-40.
23. BROADHURST, JEAN. 1915. Jour. Infect. Dis., 17-277.
24. BROWN, J. H. 1919. Monograph of the Rockefeller Inst.
25. BROWN, J. H. 1920. Jour. Exper. Med., 31-35.
26. BROWNE, WM. W. 1914. Jour. Infect. Dis., 15-580.
27. BROWN, PALFREY and HART. 1919. Jour. A. M. A., 72-7.
28. BRONFENBRENNER, J., and SCHLESINGER, M. J. 1918. Proc. Soc. Expr. Med. and Biol., 16-44.
29. BRONFENBRENNER, J., and DAVIS, C. R. 1918 *b*. Jour. Med. Res., 39-33.
30. BRUENN. Cited by Shohl and Janney. 1915.
31. BUCHANAN, R. E. 1918. Jour. Infect. Dis., 23-109.
32. BUNKER, J. W. M. 1919. Jour. Bact., 4-379.
33. BURTON, L. V., and RETTGER, L. F. 1917. Jour. Infect. Dis., 21-162.
34. BURRI, R. U., and DUGGELI, M. 1909. Centrabl. f. Bakt. I. Abt. Orig. Bd., 49, 145.
35. CHESNEY, A. M. 1916. Jour. Exper. Med., 24-387.
36. CHESTER, F. D. 1909. A Manual of Determinative Bact. New York.
37. CLARK, W. M., and LUBS, H. A. 1917 *a*. Jour. Bact., 201.
38. CLARK, W. M. and LUBS, H. A. 1917 *b*. Jour. Bact., 109.
39. CLARK, W. M., and LUBS, H. A. 1917 *c*. Jour. Bact., 191.
40. CLARK, W. M. 1922. The Determination of H-ions. (Williams & Wilkins.)
41. CLARK, W. M. 1915. Jour. Biol. Chem., 22-87.

42. CLARK, W. M., and LUBS, H. A. 1915. Jour. Infect. Dis., 17-160.
43. CLARK and LUBS. 1916. Jour. Biol. Chem., 24-479.
44. COBBET. 1897. Ann. de l'Inst. Past., 11-251.
45. COLE, S. W., and ONSLOW, E. 1916. Lancet, 191-1011.
45*a*. COLE, S. W. 1919. Practical Physiological Chem. (C. V. Mosby Co.)
46. COHEN, B., and CLARK, WM. 1919. Jour. Bact., 4-409.
47. CULLEN, G. E., and CHESNEY, A. M. 1918. Jour. Expr. Med., 28-289.
48. DAWSON, A. I. 1919. Jour. Bact., 4-133.
49. DEBORD, G. G. 1923. Jour. Bact., 8-7.
50. DERNBY, K. G., and AVERY, O. T. 1918. Jour. Expr. Med., 28-345.
51. DERNBY, K. G. 1921. Annale de l'Inst. Pst., 35-277.
52. DERNBY, K. G., and NASLUND, C. 1923. Zeit. F. Immunitatsf., 35-450.
53. DICK, H., and DICK, GLADYS. 1924. Jour. A. M. A., 82-301.
54. DOCHEZ, A. R., and GILLISPIE, L. J. 1913. Jour. A. M. A., 61-727.
55. DOCHEZ, A. R. 1924. Proc. Soc. Exp. Biol. and Med., 21-184.
56. DURHAM, H. E. 1901. Jour. Expr. Med., 5-353.
57. DUCHACEK, F. 1904 *a*. Centralbl. f. Bakt. I. Abt. Orig. Bd., 37-161.
58. DUCHACEK, F. 1904 *b*. Centralbl. f. Bakt. I. Abt. Orig. Bd., 37-326.
59. ECKER, E. E., and MEGRAIL, E. 1923. Jour. Infect. Dis., 33-269.
60. ESTY, J. R., and CATHCART, P. H. 1921. Jour. Infect. Dis., 29-29.
61. FENNEL, E. A., and FISHER, M. B. 1919. Jour. Infect. Dis., 25-444.
62. FISHER, ALBERT. 1918. Jour. Exp. Med., 28-529.
62*a*. FISHER, ALBERT. 1913. Centrl. f. Bkt. I Abt. Orig. Bd., 69-474.
63. FOLIN, O. and WU. H. 1920. Jour. Biol. Chem., 41-367.
64. FOSTER, L. F., and RANDALL, S. B. 1921 *a*. Jour. Bact. 6-143.
65. FOSTER, L. F. 1921 *b*. Jour. Bact., 6-161.
66. FRED, E. B., PETERSON, W. H., and DAVENPORT, A. 1919. Biol. Chem., 39-347.
67. FRED, E. B., and PETERSON, W. H. 1920. Jour. Infect. Dis., 27-539.
68. FULLER, G. A., and ARMSTRONG, V. A. 1913. Jour. Infect. Dis., 13-442.
69. GARBET, A. L. 1916. Jour. Immunology, 1-391.
70. GARDNER and WALKER. 1921. Jour. Hyg., 20-110.
71. GAY, F. P., and CLAYPOLE, E. F. 1913. Jour. A. M. A., 60-1141.
72. GILLESPIE, L. J. 1916. Jour. Wash. Acad. Sci., 6-7.
73. GLENN, T. H. 1911. Centralbl f. Bakt. I. Abt. Orig. Bd., 58, 481.
73*a*. GORDON, H. 1905. Lancet, 2-1400.
74. GOODALL. 1918. Lancet, 881, Dec. 28.
75. GRACE, L. G., and HIGHBERGER, F. 1920. Jour. Infect. Dis., 26-461.
76. HAJES, K. 1922. Centralbl. f. Bkt., 88-583.
77. HALL, I. W., and FRASER, A. D. 1922. Jour. Path. and Bact., 25-19.
78. HALL, I., and RANDALL, S. B. 1922. Jour. Inf. Dis., 31-326.
79. HAWN, HOPKINS and MEADER. 1918. Jour. A. M. A., 72-6.
80. HARDEN. 1901. Jour. Chem. Soc., 79-610.
81. HARTLEY and HARTLEY. 1922. Jour. Path. and Bact., 25-468.
82. HENDERSON, L. J., and WEBSTER, H. B. 1907. Jour. Med. Res., 16-1.
83. HOLMAN, W. L. 1916. Jour. Med. Res., 34-377.
84. HOOKER, S. B. 1916. Jour. Immunology, 2-1.
85. HOLT-HARRIS, and TEAGUE, O. 1916. Jour. Infect. Dis., 18-596.

85*a*. HULTON-FRANKEL, F., BARBER, H., and PYLE, E. 1919 *a*. Jour. Inf. Dis., 24-9.
86. HULTON, FRANKEL, F., and BARBER. 1919 *b*. Jour. Infect. Dis., 24-17.
87. HURTWITZ, S. K., MEYER and OSTENBERG. 1916. Johns Hopkins Bulletin 27, 16.
88. ISHII, O. 1920. Jour. Bact. V., 237.
89. IWANOW, M. S. 1892. Ann. de l'Inst. Past., 6-131.
90. JONES, F. S. 1920. Jour. Expr. Med., 32-273.
91. JONES, F. D. 1918 *a*. Jour. Expr. Med., 28-149.
92. JONES, F. D. 1918 *b*. Jour. Exper. Med., 28-253.
93. JONES, HORRY. 1920. Jour. Infect. Dis., 26-160.
94. JORDAN, E. O. 1920. Jour. Infect. Dis., 26-427.
95. JORDAN, E. O. 1917 *a*. Jour. Infect. Dis., 20-457.
96. JORDAN, E. O. 1917 *b*. Jour. Infect. Dis., 21-554.
97. JORDAN, E. O. 1918. Jour. Infect. Dis., 32-252.
98. JORDAN, E. O. 1926. Jour. A. M. A., 86-177.
99. JULIANELLE, L. A. 1922. Jour. Infect. Dis., 31-256.
100. KENDALL, A. I., DAY, A. A., and WALKER, A. W. 1913. Jour. Am. Chem. Soc., 35-1201.
101. KENDALL, A. I. 1923. Bacteriology General, Pathological, Intestinal.
102. KENDALL, A. I. 1921. Jour. Infect. Dis. 30-251.
103. KENDALL, A. I., and WALKER, A. W. 1916. Jour. Infect. Dis., 17-442.
104. KITASATO, S. 1888. Zeit. f. Hyg., 3-404.
106. KLIGLER, I. J. 1916. Jour. Bact., 1-663.
107. KOSER, S. A. 1921. Jour. Infect. Dis., 29-70.
108. KRUMWIEDE, C., KOHN, L. A., and VALENTINE, E. 1918. Jour. Med. Res. N. S., 33-89.
109. LEVINE, M. 1917. Am. Jour. Pub. Health, 7-784.
110. LEVINE, M. 1916 *a*. Jour. Inf. Dis., 19-773.
111. LEVINE, M. 1916 *b*. Jour. Bact., 1-153.
112. LORD, F. T. 1919. Jour. Expr. Med., 30-379.
113. LORD, F. T., and NYE, R. N. 1919. Jour. Expr. Med., 30-389.
114. MANTEUFEL. 1921. Centralbl. f. Bakt. I Abt. Orig. Bd., 87-160.
115. MCDONALD, E. L. 1917. Jour. Infect. Dis., 21-322.
116. MEYER, K. F. 1921. Jour. Infect. Dis. 28-381.
116*a*. MICHAELIS, L., and MARCORA, P. 1912. Zeit. f. Im. I. Abt. Orig., 14-170.
117. MOON, V. H. 1914. Jour. Infect. Dis., 14-56.
118. MORISHIMA, K. 1921. Jour. Bact., 6-275.
119. MORISHIMA, K., and TEAGUE, O. 1921. Jour. Inf. Dis., 28-145.
120. MOCK. 1919. Jour. Lab. and Clin. Med., 5-54.
121. MULLER, REINER. 1911. Centralbl. f. Bakt. I. Abt. Og. Bd., 58-97.
122. MULLER, L. 1896. Centralbl f. Bakt. I. Abt. Orig. Bd., 53-209.
123. MYER, K. F., NELSON, N. M., and FENSIER, M. L. 1921. Jour. Infect. Dis., 28-408.
124. NEUFELD, F., and HAENDEL. 1910. Cited by Avery, 1915.
125. OPPENHEIM, C. J. 1920. Jour. Infect. Dis., 26-117.
126. PENFOLD, W. J. 1911. Jour. Hyg., 11-30.
127. PENFOLD, W. J. 1912. Jour. Hyg., 12-195.
128. PERE, M. A. 1892. Ann. l'Inst. Past. 6-512.
129. RAMBOUSEK, J. 1900. Zeit. f. Immun. Bd. 38, p-383.

130. RAUBITSCHEK, H., and NATIONEK, D. 1913. Centralbl. f. Bakt. I. Orig. Bd., 69-241.
131. RAHN, O. 1906. Centralbl f. Bakt. II, 16, 417.
132. ROBINSON. 1915. Jour. Med. Res., 32-399.
133. ROTKY. 1921. Centralbl f. Bakt. I Abt. O. Bd., 87-16.
134. SALTER, R. C. 1919. Jour. Infect. Dis., 24-260.
135. SEARS, H. J. 1917. Jour. Bact., 2-187.
135a. SCHOENHOLZ, P., and MEYER, K. F. 1921. Jour. Infect. Dis., 28-384.
136. SERA, Y. 1910. Zeit. F. Hyg. u. Infect., 66-141.
137. SHELLER, Robt. 1908. Centralbl f. Bakt. I. Orig. Bd. 46-385.
138. SHERWOOD, N. P., DOWNS, C. M., and MCNAUGHT, J. B. 1920. Jour. Infect. Dis., 26-16.
139. SHERWOOD, N. P. 1926. Kan. Sc. Bull. XVI.
139a. SHOHL, A. T., and JANNEY, J. H. 1917. Jour. Urology, 1-211.
140. SIMONDS, J. P. 1915. Jour. Infect. Dis., 17-500.
141. SMILLIE, W. C. 1917. Jour. Infect. Dis., 20-45.
142. STOBER. 1904. Jour. Infect. Dis., 1-445.
143. TEAGUE, O., and MORISHIMA, K. 1920. Jour. Infect. Dis., 26-52.
144. TEAGUE, O., and MCWILLIAMS, H. I. 1917. Jour. Immunology, 2-383.
145. TEN-BROECK, C. 1916. Jour. Expr. Med., 24-213.
146. THRO, W. C. 1914. Jour. Infect. Dis., 15-234.
147. THRO, W. C. 1915. Jour. Infect. Dis., 17-227.
148. TISSIER, H., and MARTELLY. 1902. Ann. de l'Inst. Past., 16-865.
149. TEAGUE, O., and TORREY, J. C. 1907. Jour. Med. Res., 16-329.
150. TORREY, J. C., and BUCKELL, GEO. T. 1922. Jour. Immunology, 7-305.
151. TOYODA, H. 1922. Centralbl f. Bakt. I. Abt. O. Bd., 88-539.
152. TREECE, E. L. 1920. Abstracts of Bact., 4, 9.
153. TROWBRIDGE, FINKLE and BARNARD. 1915. Jour. A. M. A., 64-728.
154. TWORT. 1907. Proc. Roy. Soc., 79-329.
155. VAUGHN. 1919. Jour. Lab. and Clin. Med., 4-640.
156. WADE and MCDANIELS. 1915. Am. Jour. Pub. Health, 5-136.
157. WEISS, H. 1917. Jour. Med. Res., 36-135.
158. WINSLOW, C. E. A., and LOCKRIDGE, E. E. 1906. Jour. Inf. Dis., 3-549.
159. WINSLOW, C. E. A., KLIGLER, I. J., and ROTHBURG, W. 1919. Jour. Bact. 4-429.
160. WRIGHT, J. H. 1917. Jour. Bact., 2-315.
161. YURI. 1923. Jour. Infect. Dis., 32-478.
162. ZINSSER. 1923. Infection and Resistance. (Macmillan Co., N. Y.)

ACKNOWLEDGMENT.

I wish to express my sincere appreciation to Dr. N. P. Sherwood, chairman of the Department of Bacteriology, who has made it possible for me to complete the work required for the degree of Doctor of Philosophy. He has given constant encouragement and invaluable advice.

Thanks is also due the Research Committee of the University of Kansas for much of the equipment which has made it possible to complete this work.